

# How new biopharmaceuticals are developed: A swedish perspective



[www.affibody.com](http://www.affibody.com)

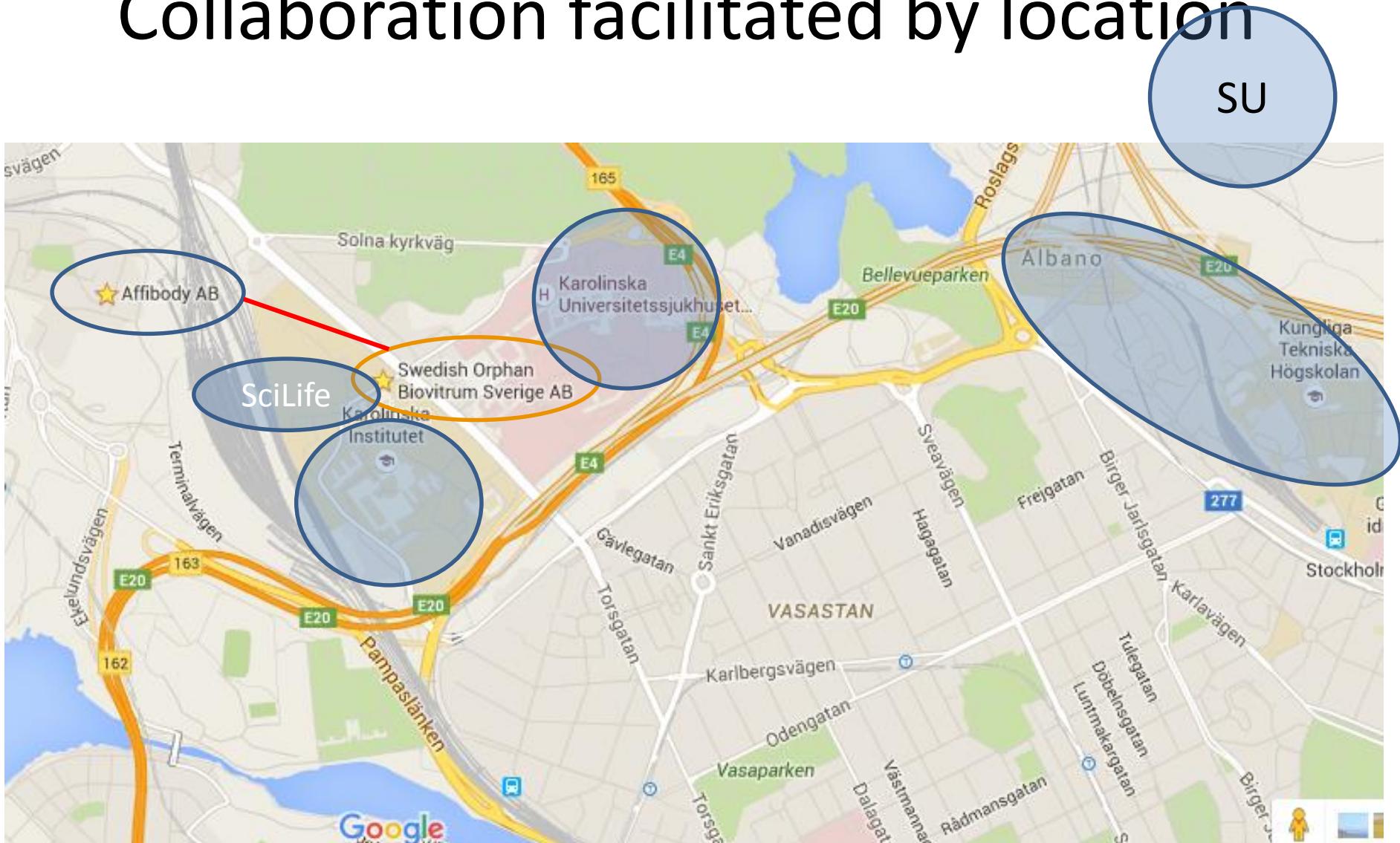
Fredrik Frejd,  
Research

Sep 13, 2017



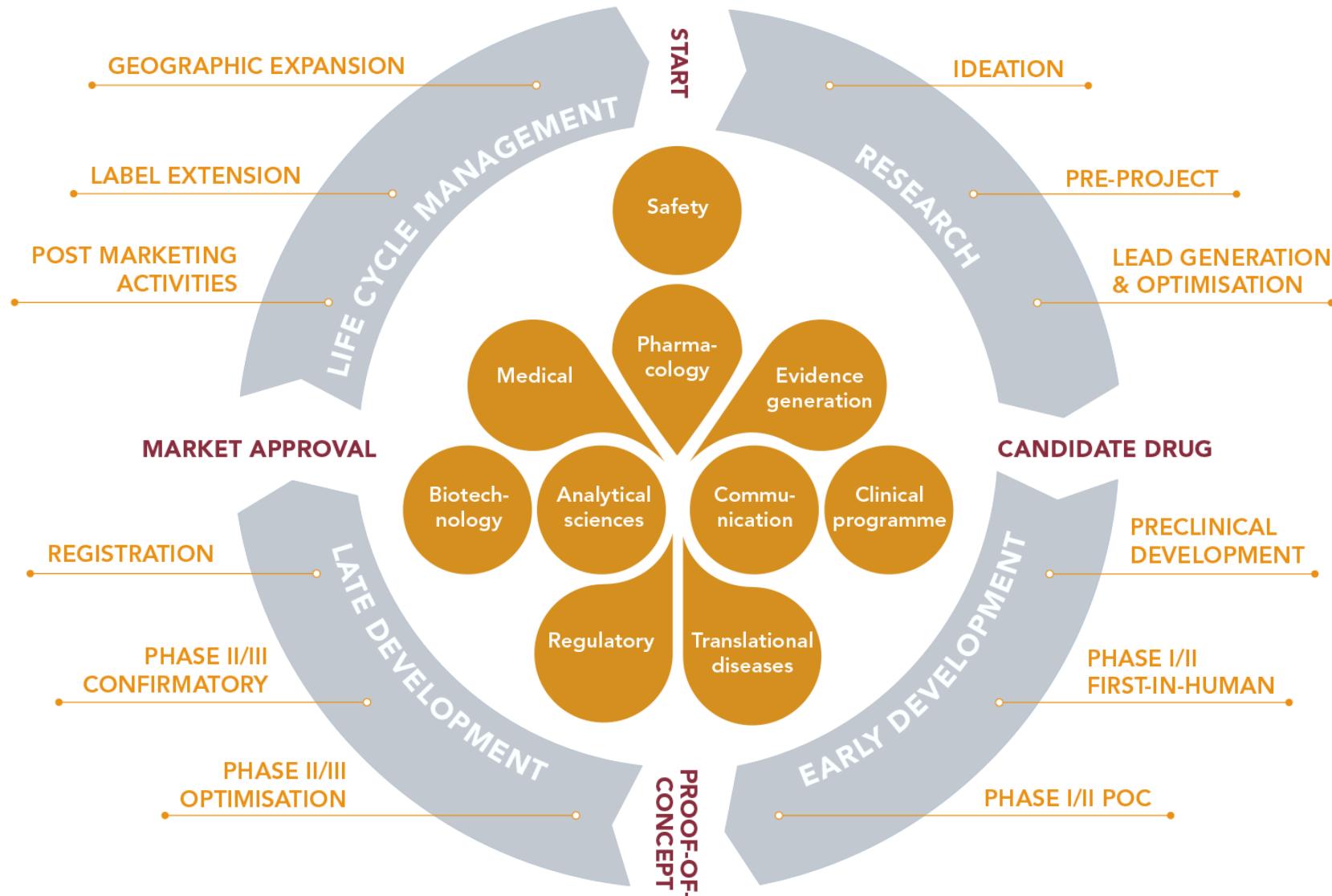
Erik Nordling,  
Biomedical Science &  
Portfolio Innovation

# Collaboration facilitated by location

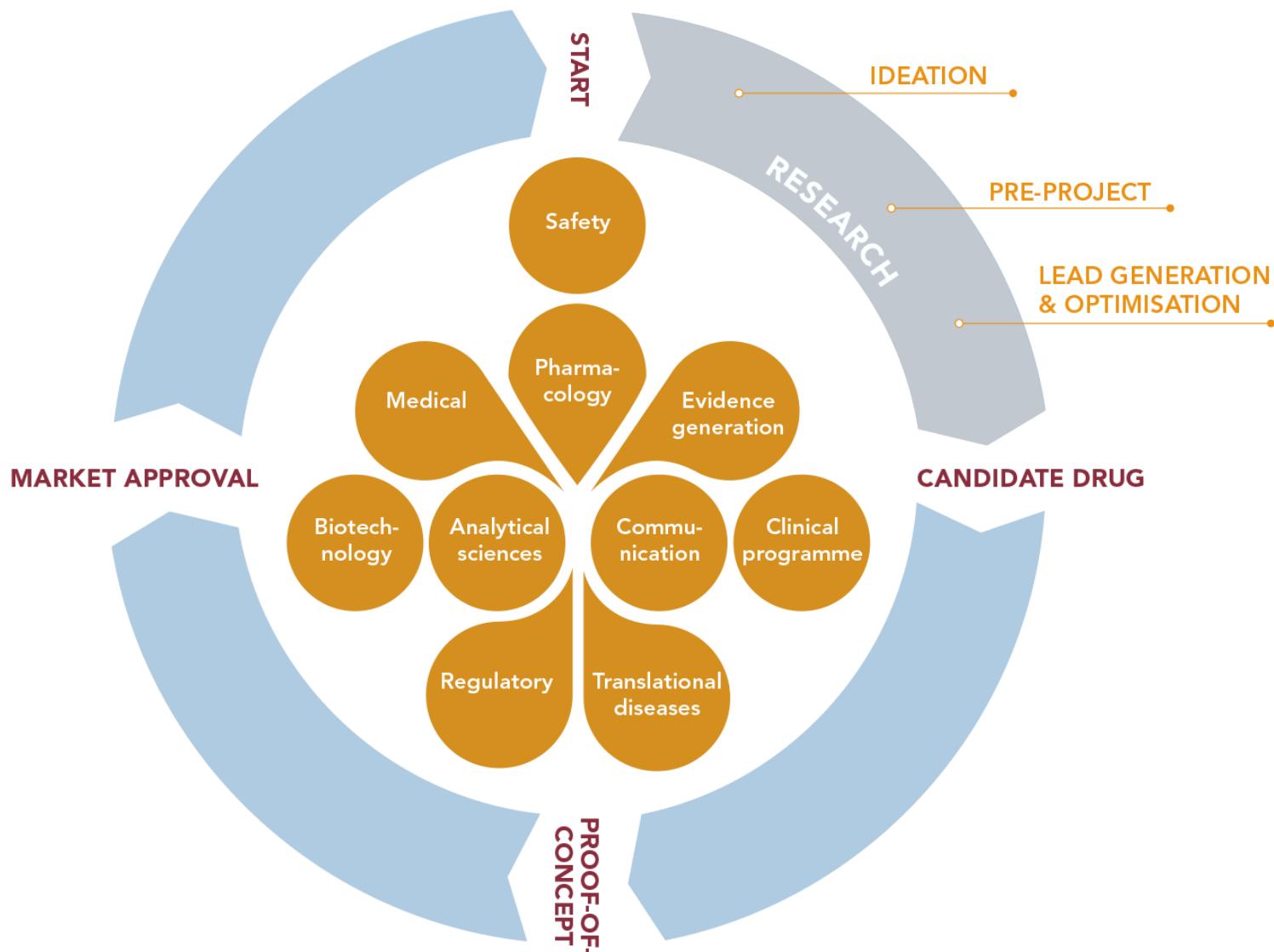


Life science cluster with major schools (KTH, KI, SU) 2

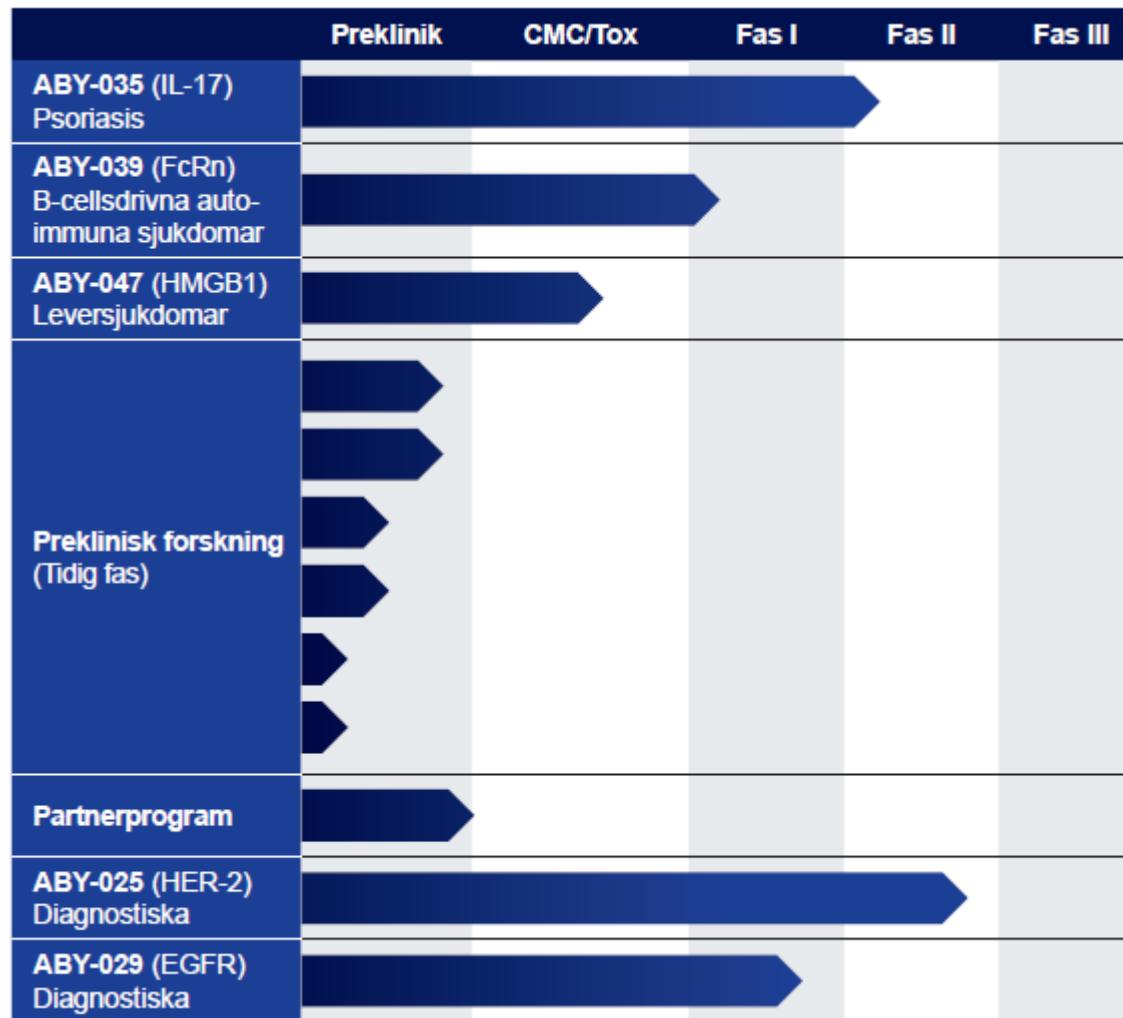
# Drug development life cycle



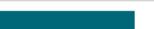
# Drug development life cycle



# Affibody pipeline



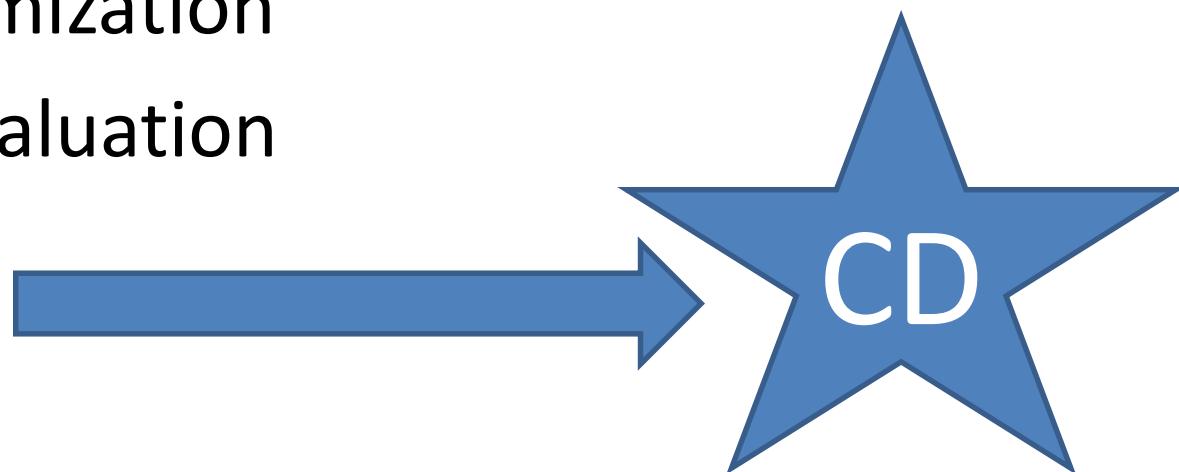
# Sobi pipeline

Therapeutic area/Indication	Product/Project	Pre-clinical	Phase 1	Phase 2	Phase 3	Phase 4
Haemophilia A	Elocta/A-SPIRE					
Haemophilia A	Elocta/PUP A					
Haemophilia A	XTEN*/BIVV001					
Haemophilia A	Elocta/ASURE					
Haemophilia A	Elocta/reITrate					
Haemophilia A	Elocta/verIT18					
Haemophilia A and B	Elocta/ Alprolix/PREVENT					
Haemophilia B	Alprolix/B-YOND					
Haemophilia B	Alprolix/PUP B					
Haemophilia B	XTEN*/BIVV002					
Acute gout	Kineret/anaGO					
Still's disease	Kineret/anaSTILLS					
Alkaptonuria	Orfadin/SONIA2					
MPS IIIA	SOBI003					
Anti-C5	SOBI005					
Anti-IL-1	SOBI006					

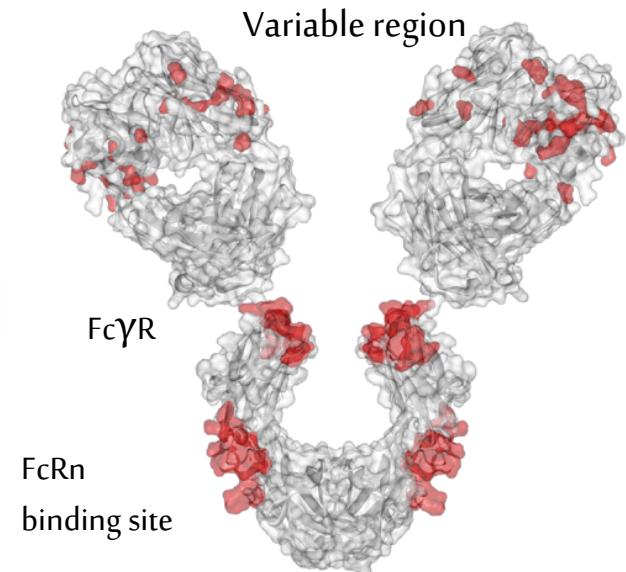
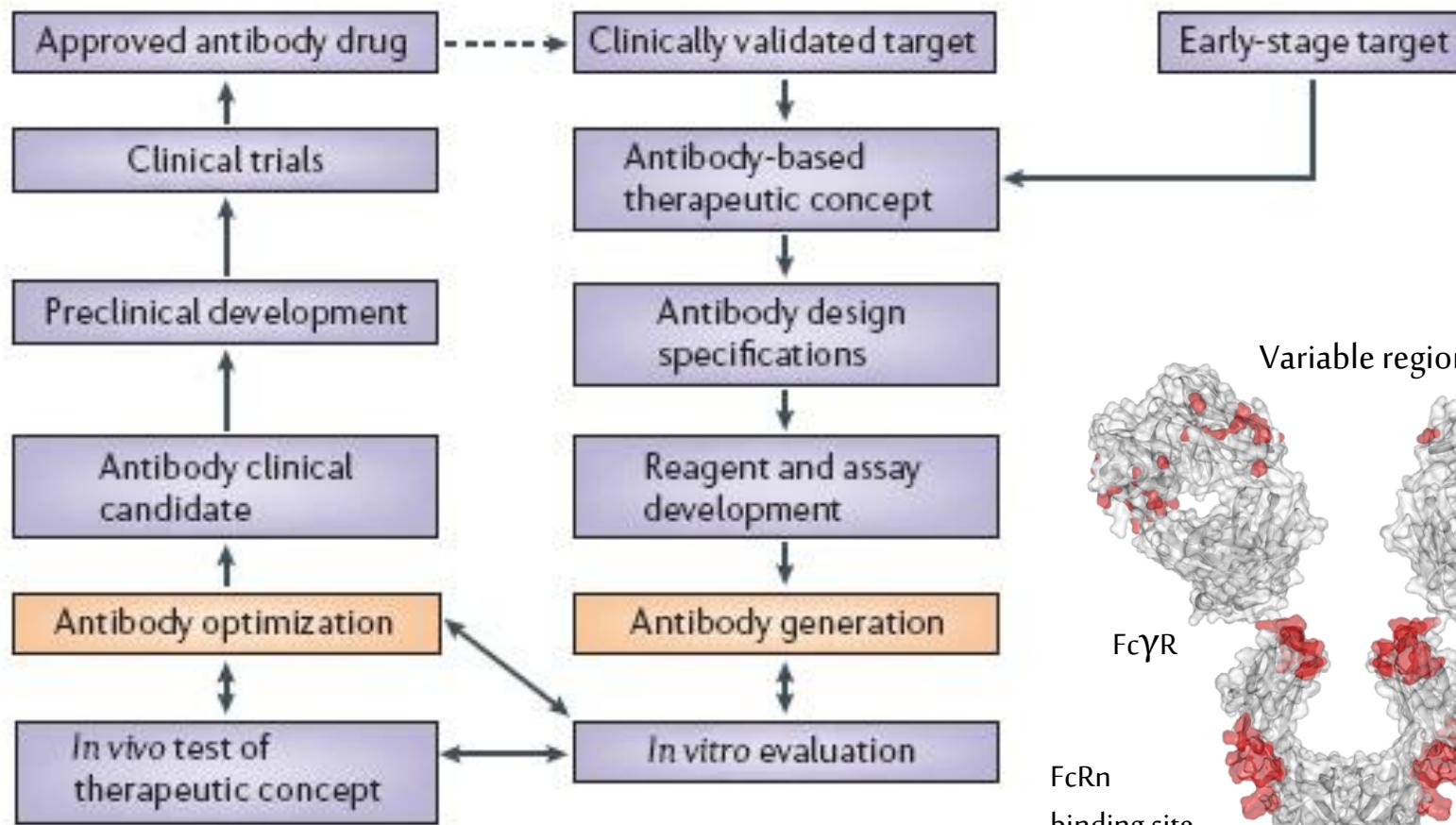
9 \* XTEN are Bioverativ development program, Sobi has elected to add programs to the collaboration agreement but not yet opted-in

# Introduction: Discovery/Research

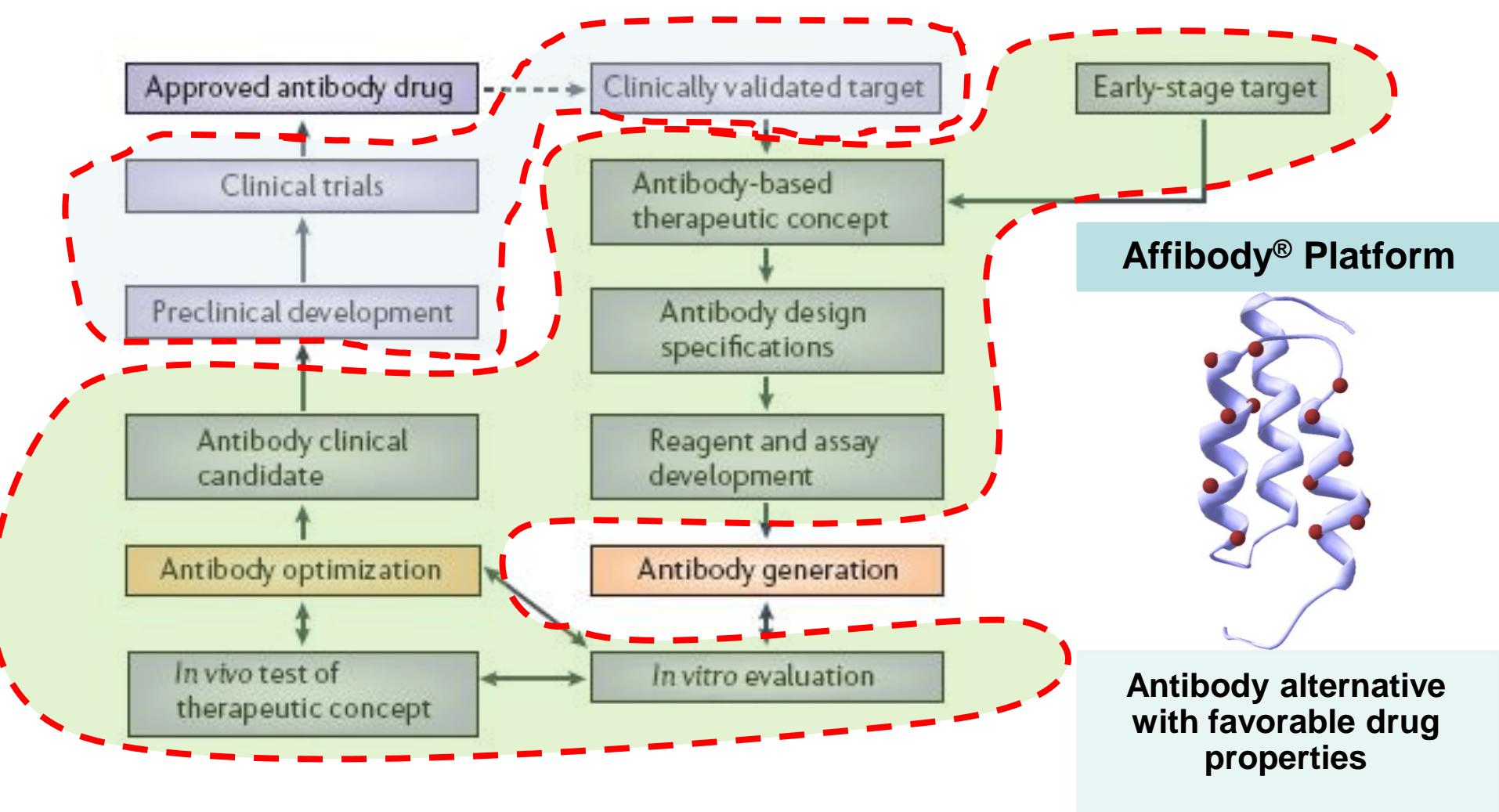
- Choose Indication
- What target biology
- Target analysis
- Lead discovery
- Lead optimization
- Pre-CD evaluation



# Process for Antibody Drug Discovery



# Process for Antibody Drug Discovery



# Pre-study document

## XCXCXC



Date: 140528

Responsible Authors: Lindvi Gudmundsdotter and Susanne Klint

Project Team: Lindvi, Susanne, Fredrik, Anders, and Ingmarie

## SUMMARY OF THE XCXCXC PRESTUDY – MAIN ISSUES AND CONCERNS

- PROJECT GOAL
- PROJECT BACKGROUND
- TECHNICAL FEASIBILITY
- PHARMACOLOGY
- COMPETITIVE landscape
- MARKET analysis
- CLINICAL TRIAL DESIGN
- COLLABORATION(S)
- RISK ASSESSMENT
- REFERENCES

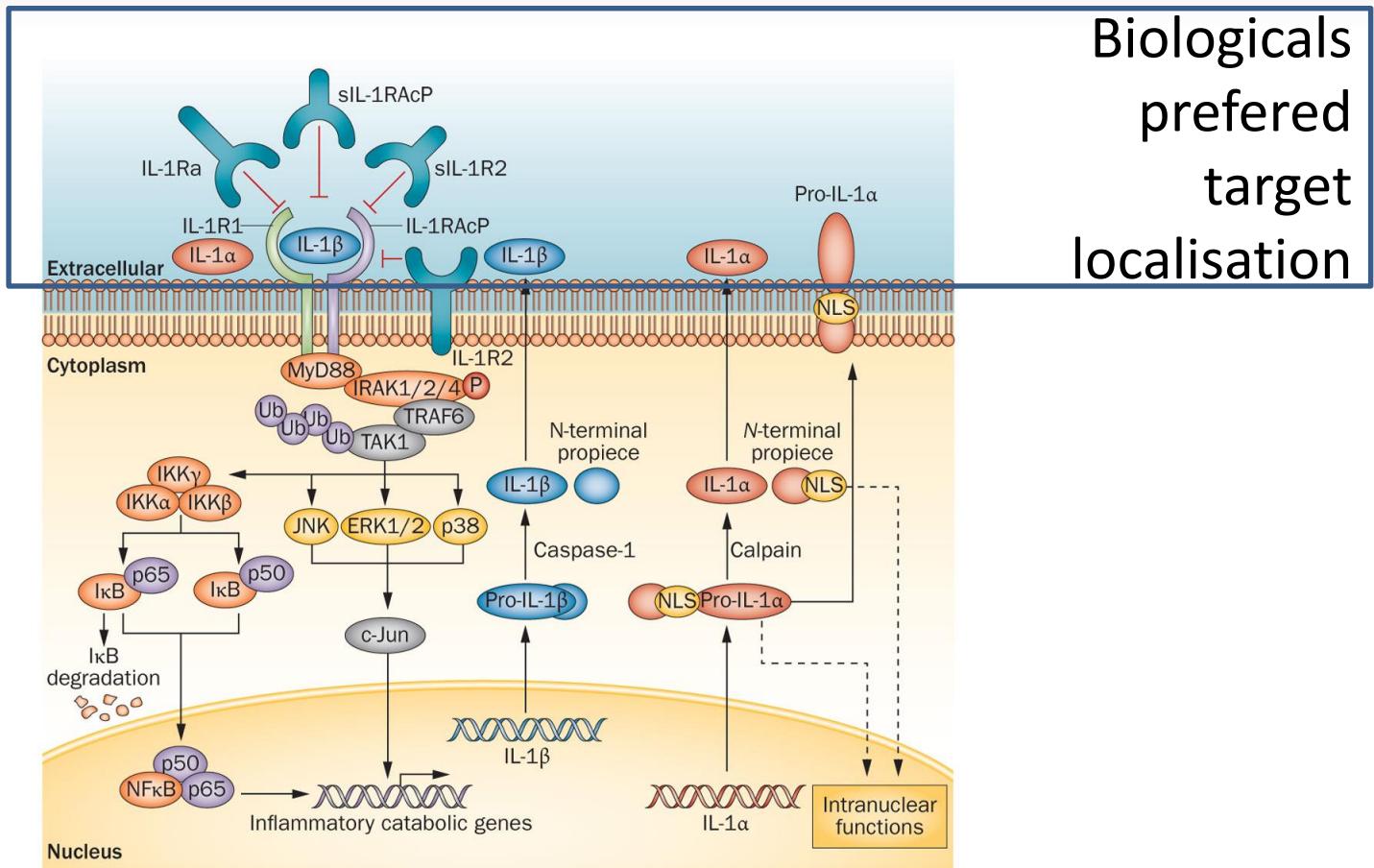
2 Case studies:  
• IL-1  
• C5

# CASE 1 IL-1 Biology-Modality

- IL1-RA : Kineret
- IL-1 trap: Arcalyst
- anti-IL1-mab: Illaris
- allosteric mAb: Gevokizumab (XOMA)
- hybrid IL-1RA/IL-1beta: Eleven Biotherapeutics
- small scaffold: Affibody



**Figure 2 IL-1 $\alpha$  and IL-1 $\beta$  synthesis and signal transduction pathway**



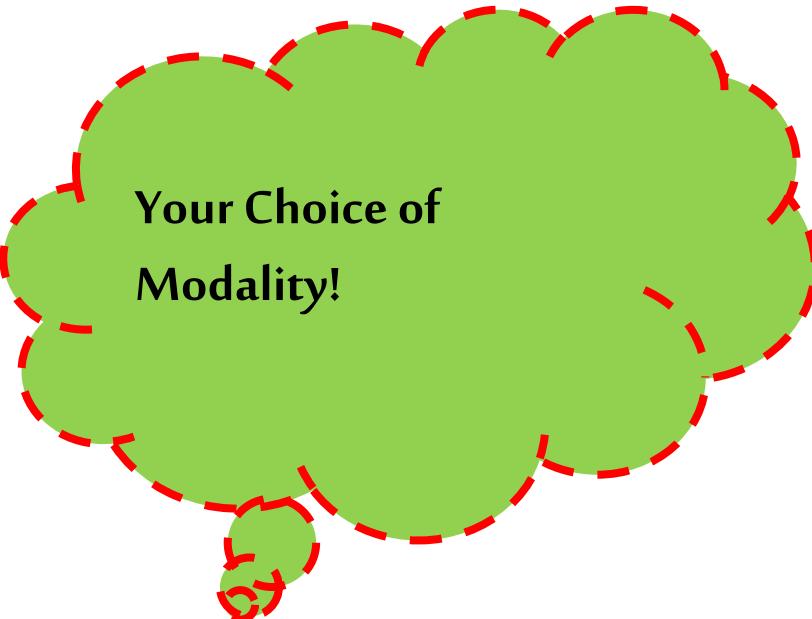
Risbud, M. V. & Shapiro, I. M. (2013) Role of cytokines in intervertebral disc degeneration: pain and disc content

*Nat. Rev. Rheumatol.* doi:10.1038/nrrheum.2013.160

Natural receptor antagonist

Engineered  
receptor antagonist

Receptor-trap



Your Choice of  
Modality!

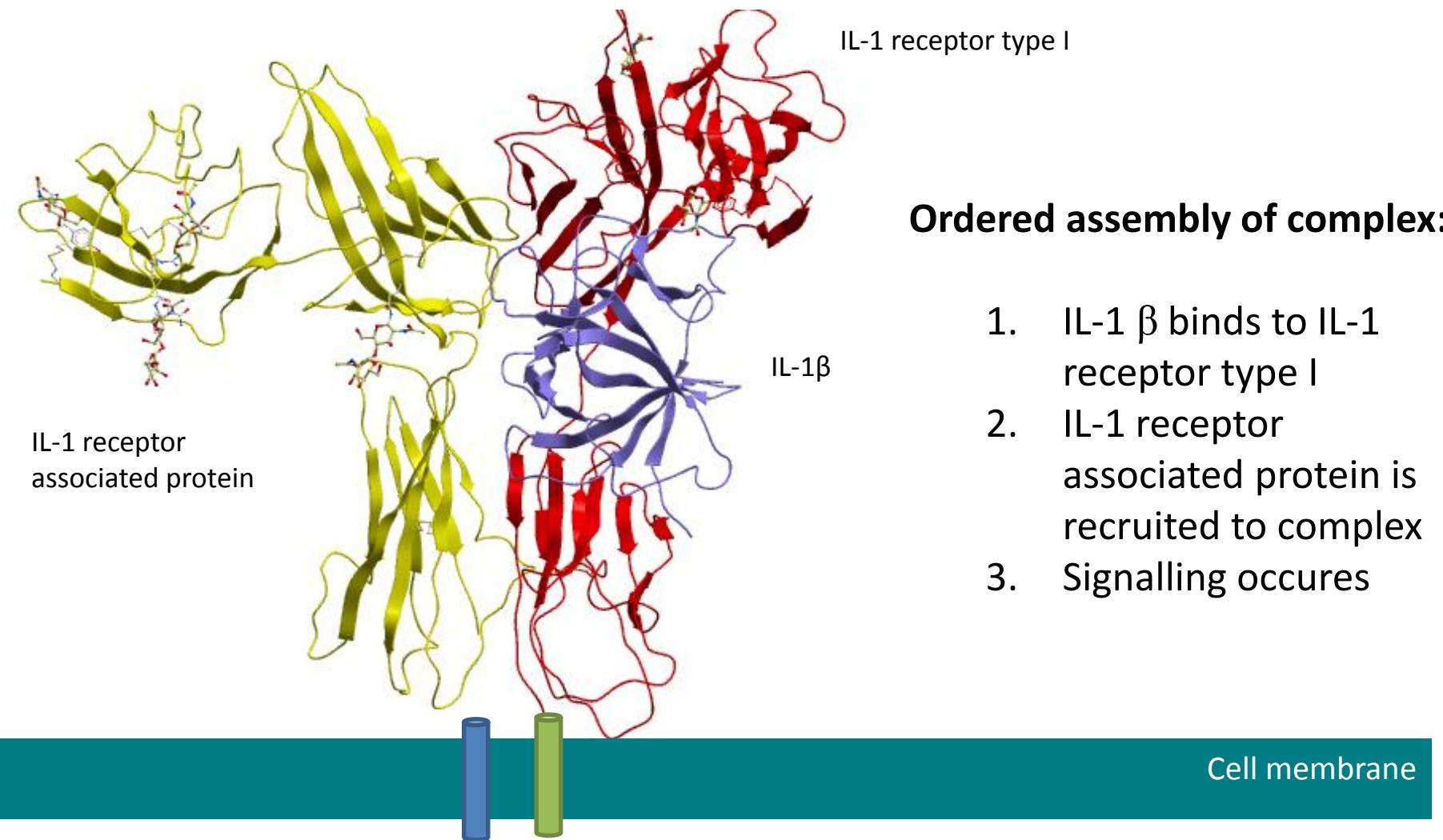
Monoclonal

Small antibody mimetic

# Target analysis

- Biologicals are well suited to disrupt protein interactions
- Biologicals are seldom hampered by off-target effects
  - Toxicity is generally mechanism related
- Cross species reactivity are not always the case

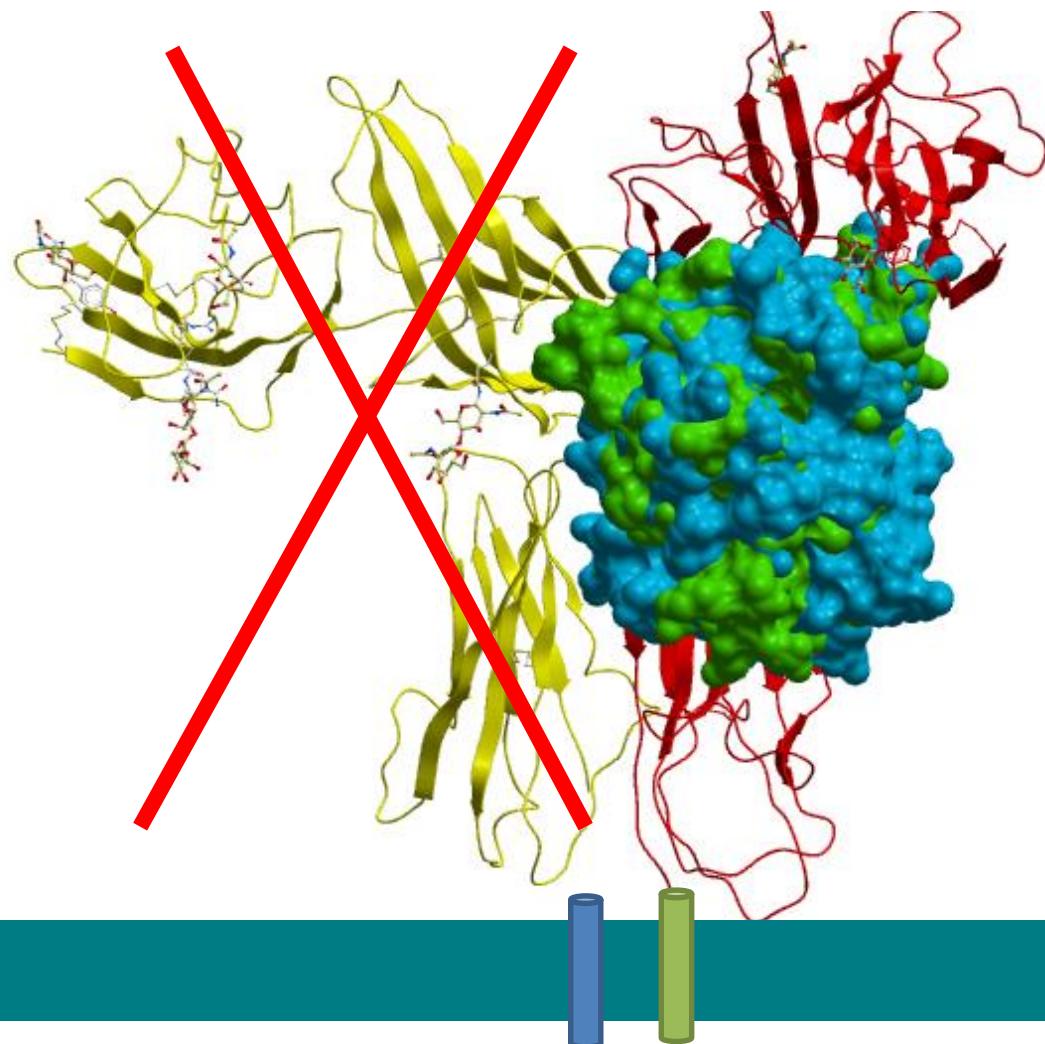
# Target analysis: IL-1 signalling complex



## Ordered assembly of complex:

1. IL-1 $\beta$  binds to IL-1 receptor type I
2. IL-1 receptor associated protein is recruited to complex
3. Signalling occurs

# Overlay with natural inhibitor IL-1Ra

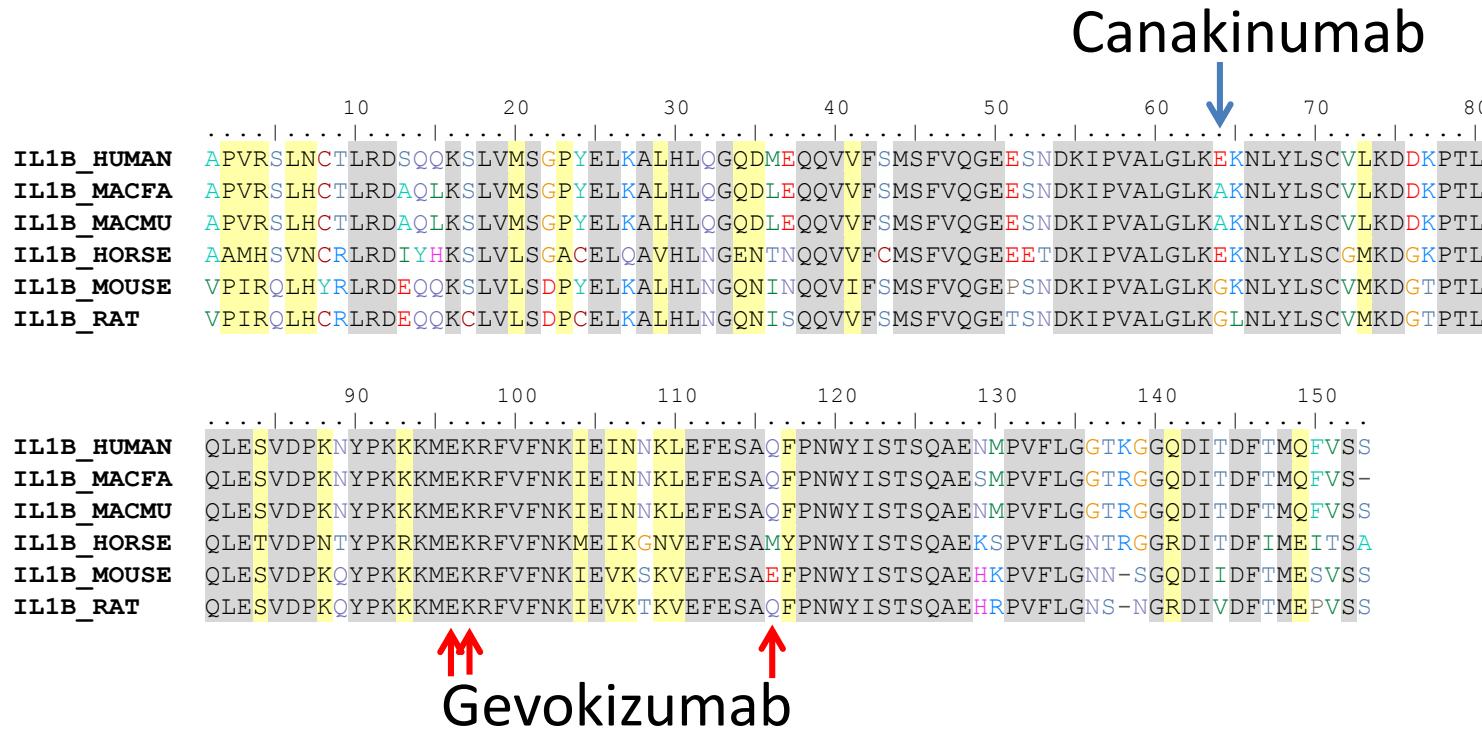


## Mechanism of action:

- Steric inhibition of IL-1Ra
- Complex can not be formed when IL-1Ra binds to IL-1 receptor type II

PDB: 3O4O; 1IRA

# Cross species activity: IL-1beta species variants and binding epitopes



- Canakinumab binds only to human IL-1b
  - Alignment suggest Horse reactivity possible
- Gevikozumab reacts with all except horse and mouse

# Likelyhood of off-target effects, biologicals vs small molecules

## Biologicals

- Identity within family 18-30 %
  - Off target effects are not likely
- Identity between Human and Mouse IL-1Rec I 63%
  - Cross-species activity are not trivial to achieve

## Small molecules

- Many ligands are similar and thus are the active sites similar
  - Off target effects likely
- Cross-species activity are common as the ligand is the same

	IL1R1_HUMAN	IL1AP_HUMAN	IL1R2_HUMAN	IL18R_HUMAN	I18RA_HUMAN	ILRL1_HUMAN	ILRL2_HUMAN	IRPL1_HUMAN	IRPL2_HUMAN
IL1R1_HUMAN	ID	0,18	0,26	0,19	0,18	0,2	0,27	0,23	0,24
IL1AP_HUMAN		ID	0,19	0,19	0,15	0,2	0,16	0,25	0,27
IL1R2_HUMAN			ID	0,16	0,18	0,19	0,24	0,2	0,22
IL18R_HUMAN				ID	0,14	0,19	0,16	0,17	0,17
I18RA_HUMAN					ID	0,16	0,16	0,13	0,17
ILRL1_HUMAN						ID	0,19	0,18	0,19
ILRL2_HUMAN							ID	0,19	0,19
IRPL1_HUMAN								ID	0,63
IRPL2_HUMAN									ID

IL-1 receptor type 1

IL-1 receptor accessory protein

IL-1 receptor type 2

IL-18 receptor 1

IL-18 accessory protein

IL-33 receptor

IL-36 receptor

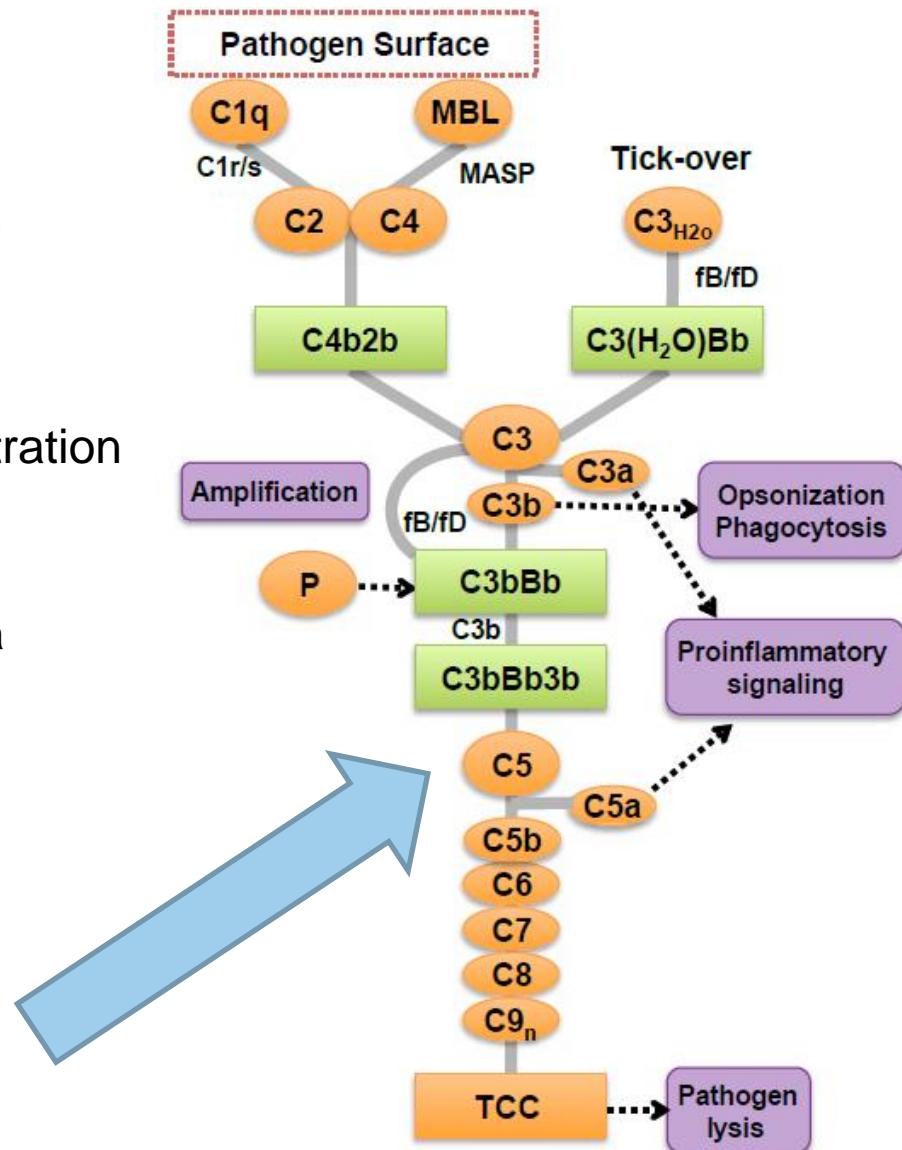
IL-1 receptor accessory protein-like 1

IL-1 receptor accessory protein-like 2

# CASE 2: Complement Biology

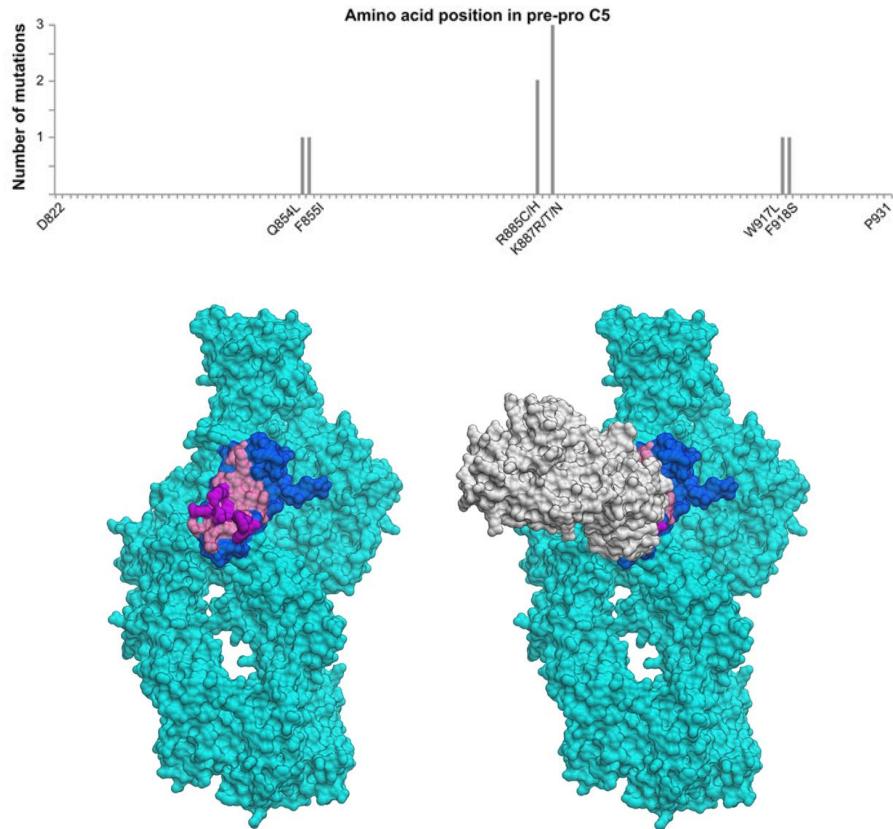
## C5 Inhibition – Rationale

- C5 is a highly attractive target
  - Common to all complement pathways
  - Blocks terminal complement
  - Proximal complement intact
- Target present at very high concentration
  - 400 nM
  - Difficult antibody target – Infusions
  - Approved antibody – Soliris, dose is a gram/2weeks



**SOLIRIS®**  
 (eculizumab)  
 Concentrated solution for intravenous infusion

# Structural epitope of Eculizumab

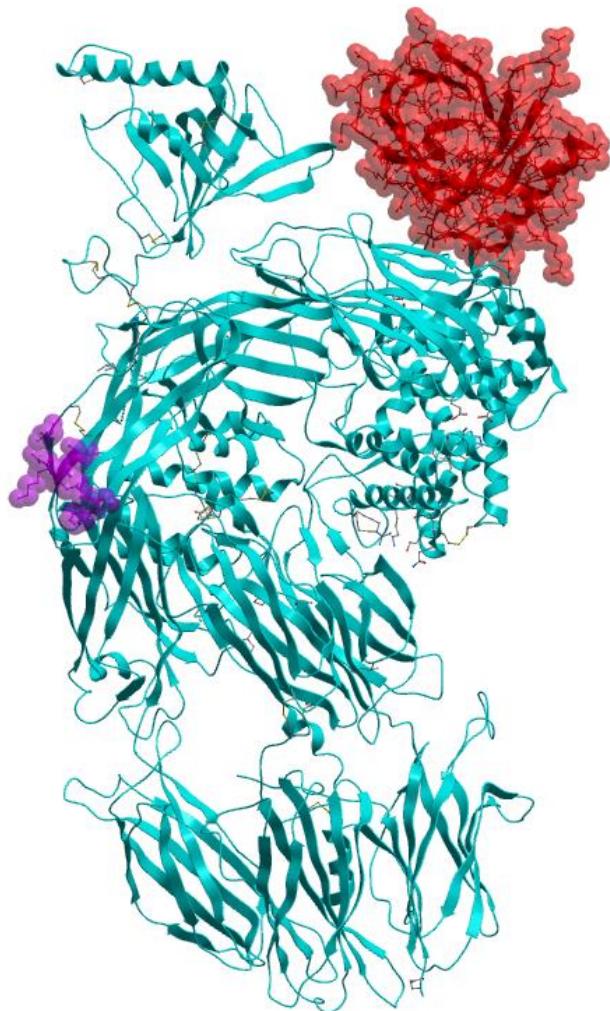


- Illustration of the six amino acid residues identified to be involved in eculizumab binding
- Visualization of the mutation sites on the structure of complete C5 (PDB5I5K).
  - The mutated residues are highlighted in magenta
  - the MG7 domain in blue
  - residues within 5 Å distance from eculizumab as from PDB5I5K are highlighted in rose.
- Illustration of the eculizumab Fab-fragment (white) bound to C5 (cyan)

- Stratification of responders towards eculizumab using a structural epitope mapping strategy, Volk *et al.* Scientific Reports 6, 31365 (2016)
- Structural Basis for Eculizumab-Mediated Inhibition of the Complement Terminal Pathway. Schatz-Jakobsen *et al.* J Immunol. 197:337-44 (2016)

# Nature guided cross species specificity

- OmCl is a complement inhibitor from the soft tick *Ornithodoros moubata*
  - Mans and Ribeiro - Insect Biochem. Mol. Biol. 2008
- Exhibit broad species selectivity
  - Useful as a tool to direct selection process towards a broadly conserved epitope
- Binding site is located in MG8, CUB, C5d domain interface
  - Structural basis for therapeutic inhibition of complement C5. Jore et al. Nat. Struct. Mol. Biol. 23: 378-386 (**2016**)



# Conclusion:

## Biopharmaceuticals vs small molecules

- Different Tox required for the two approaches
  - Biopharmaceutical require mechanism related tox program and immunogenicity assessment
  - Small molecules require general tox program that assesses both mechanism related toxicity and off-target toxicity as off-target effects are likely
    - This can also include the metabolites of the drug as well

# Lead Generation

- Aim: Complement blocking drug
  - Block protein interactions
- C5 concentration in plasma 400 nM
- Small size allows for high dose in small volume
- Small protein scaffold needed

# Subcutaneous Injection Volume and Size

- S.c. injection volume restricted to approx. 1-1.5 ml
- The molar amount of a smaller protein will be superior, at a given protein conc.

**mAb**

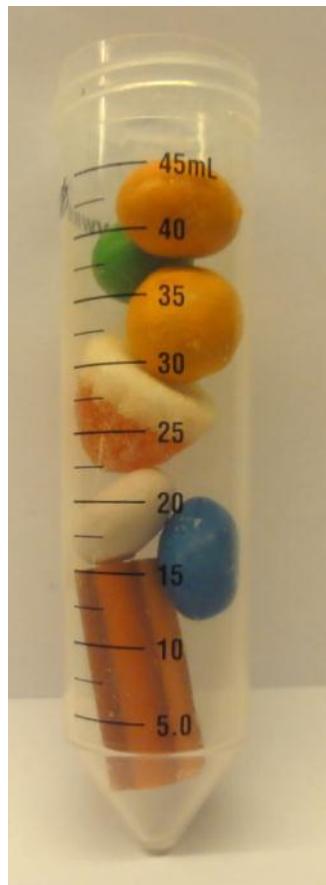
**150'000 g/mol**

**Conc.**

150 mg/mL

**COGs**

5 g / L    Mammalian  
cell culture



**Z-ABD**

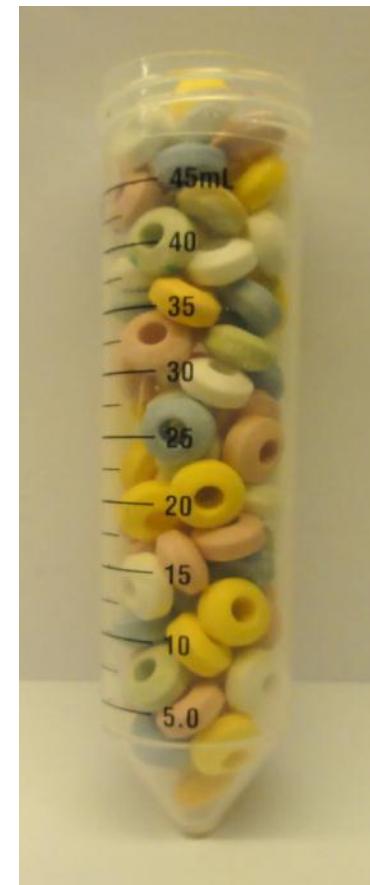
**15'000 g/mol**

**Conc.**

15 mg/mL

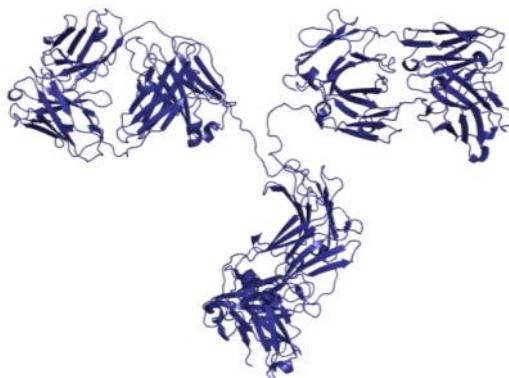
**COGs**

0.5 g / L bacterial  
fermentation



# Affibody – Combining Properties of Two Worlds

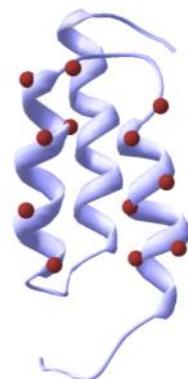
## Biopharmaceutical



### Monoclonal Antibodies

- High specificity
- High COGS
- Size: 150 kDa

## Next generation therapeutics



### Affibody® Molecules

- High specificity
- Low COGS
- Size: 6.5 kDa

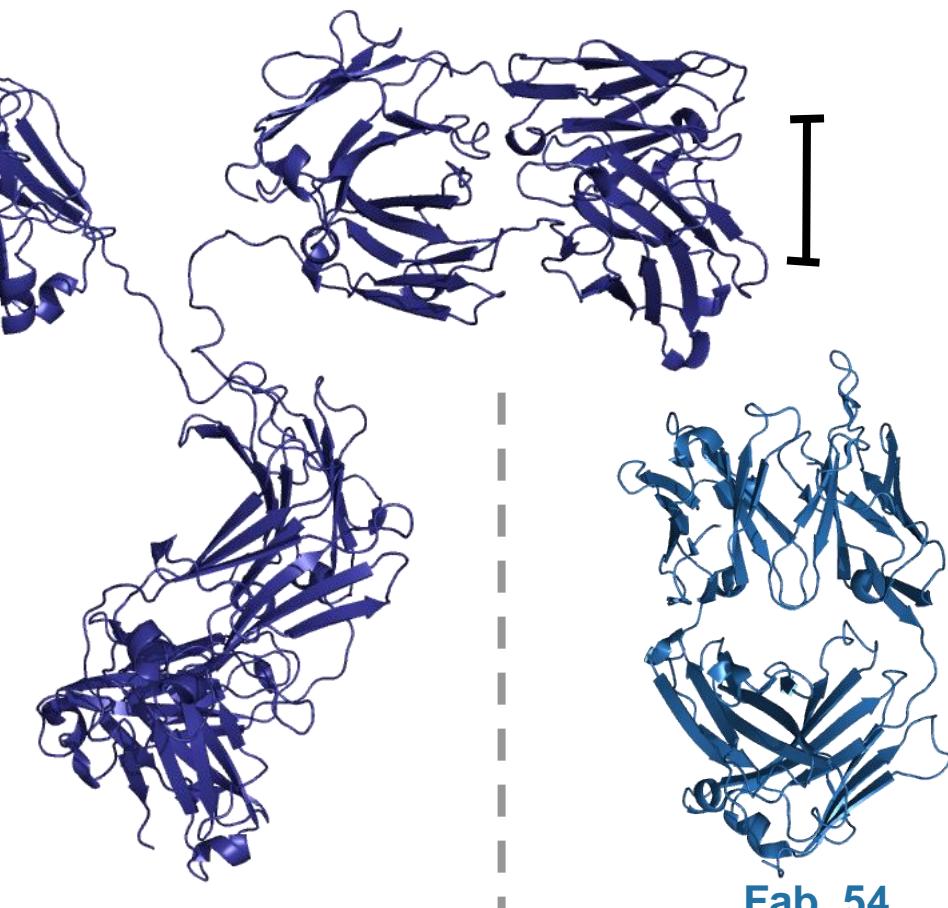
## Traditional pharmaceuticals



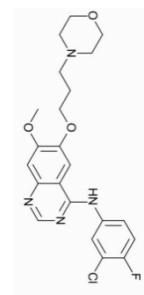
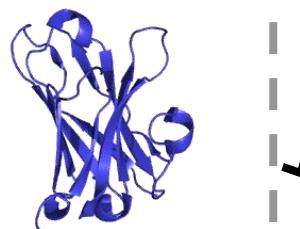
### Small Molecules

- Low specificity
- Low COGS
- Size: <0.5 kDa

# Different Recognition Molecules



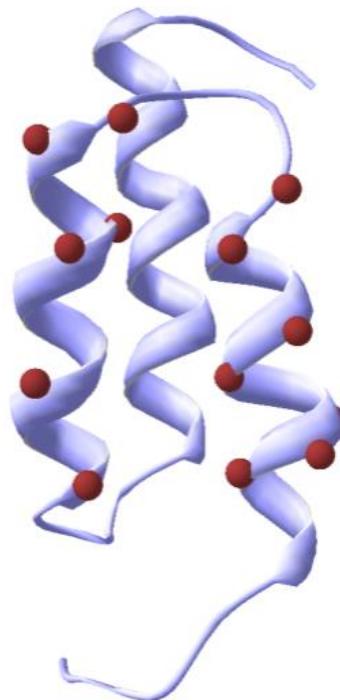
Affibody® molecule –  
A peptide with a  
defined structure



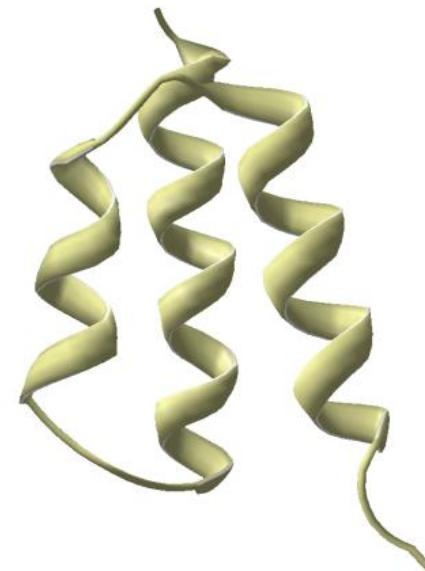
- Slow distribution
- Slow clearance
- Rapid clearance
- Rapid distribution
- Good tissue penetration

# Affibody Technology – Affibody® and Albumod™ Platforms

## Affibody® Platform



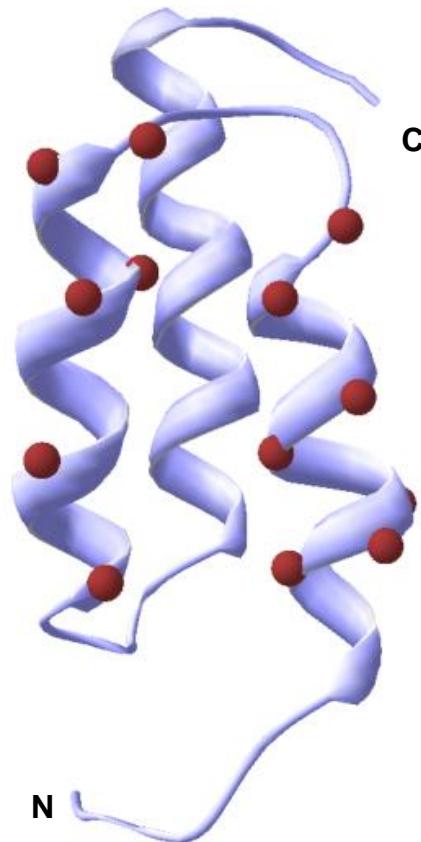
## Albumod™ Platform



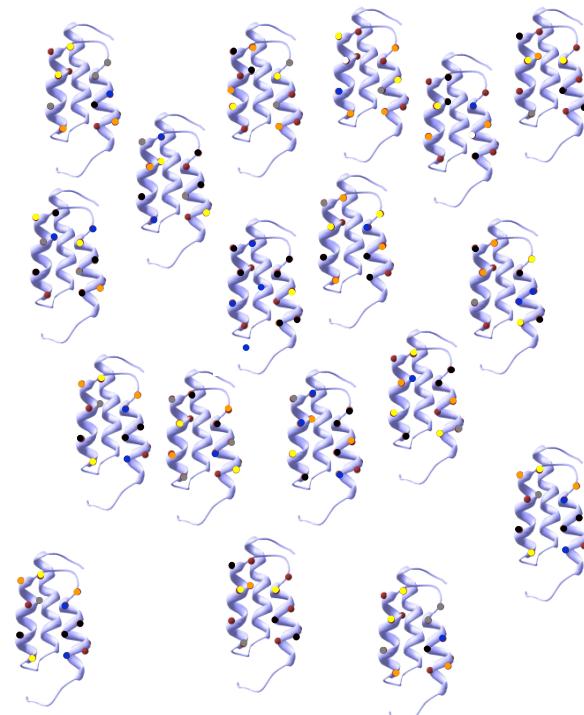
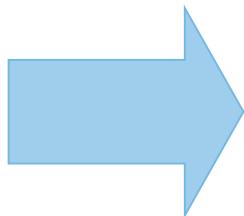
- Antibody alternative with favorable drug properties
- IP protection until 2028

- Extending the half life of biotherapeutics
- IP protection until 2030

# Molecular Diversity

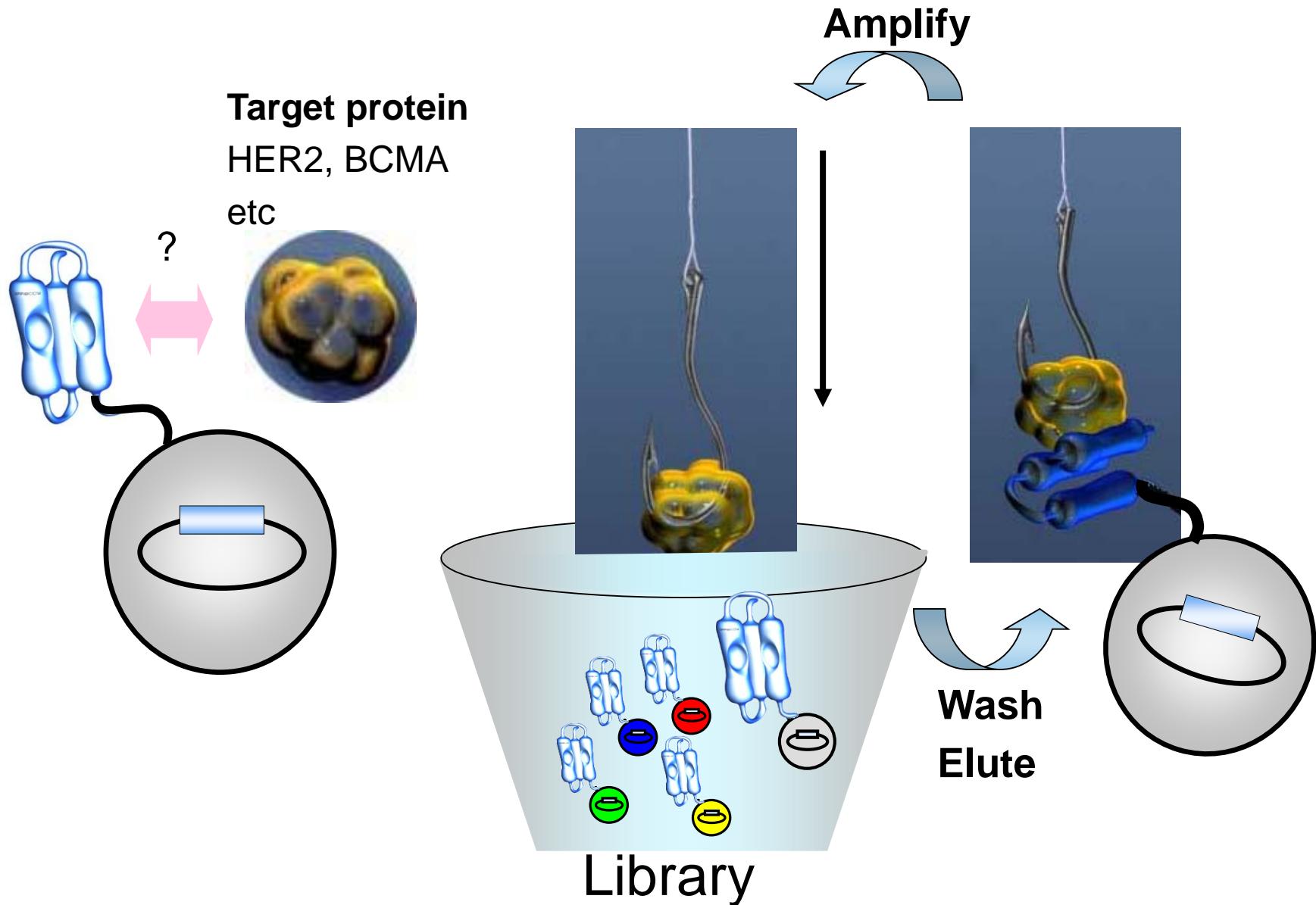


Randomization of 13  
selected positions

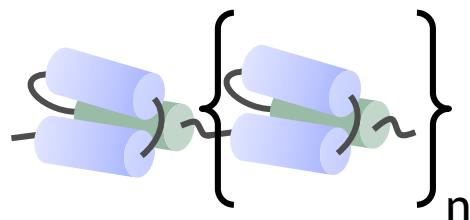


Highly functional library  
 $3 \times 10^{10}$  variants

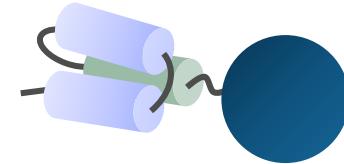
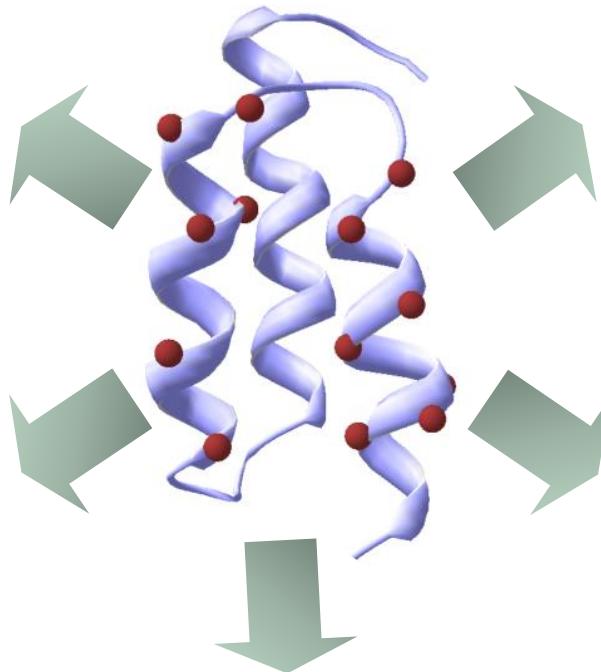
# Overview Phage Display



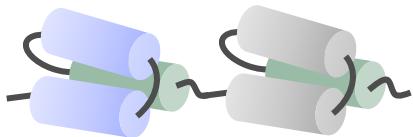
# Affibody® Molecules – Flexible Engineering



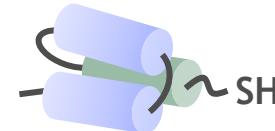
**Multimeric**  
Steffen *et al*, 2005  
Nordberg *et al* 2008  
Jonsson *et al* 2009



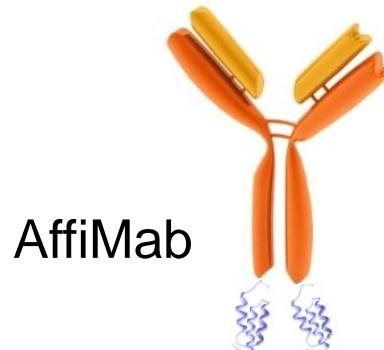
**Fusion protein**  
Rönnmark *et al*, 2004  
Tolmachev *et al*, 2007  
Zielinski *et al*, 2009, 2011



**Bi-specific**  
Friedman *et al* 2009  
Ekerljung *et al* 2012  
Malm *et al* 2014



**Site-specific modification**  
Mume, E. *et al.* 2006  
Kramer-Marek *et al* 2008  
Tolmachev *et al* 2014

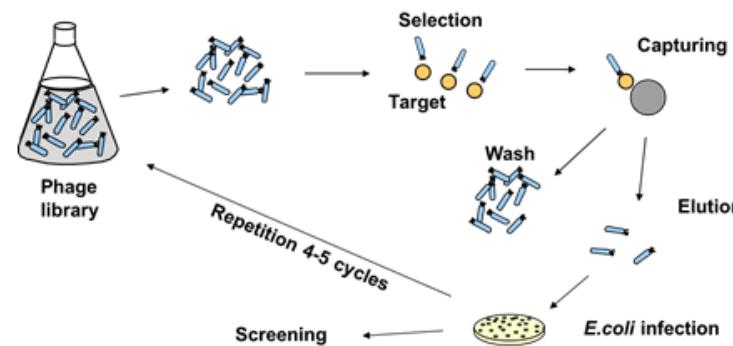


**AffiMab**

# Generation of Affibody C5 Inhibitors

## i. Selection by phage display

- Human C5 as target protein



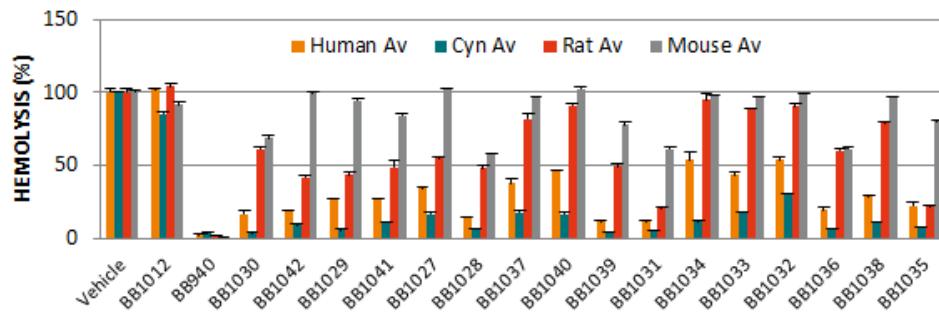
## ii. Screening for target binding

- ELISAs, competition assay etc.



## ii. Functional screening

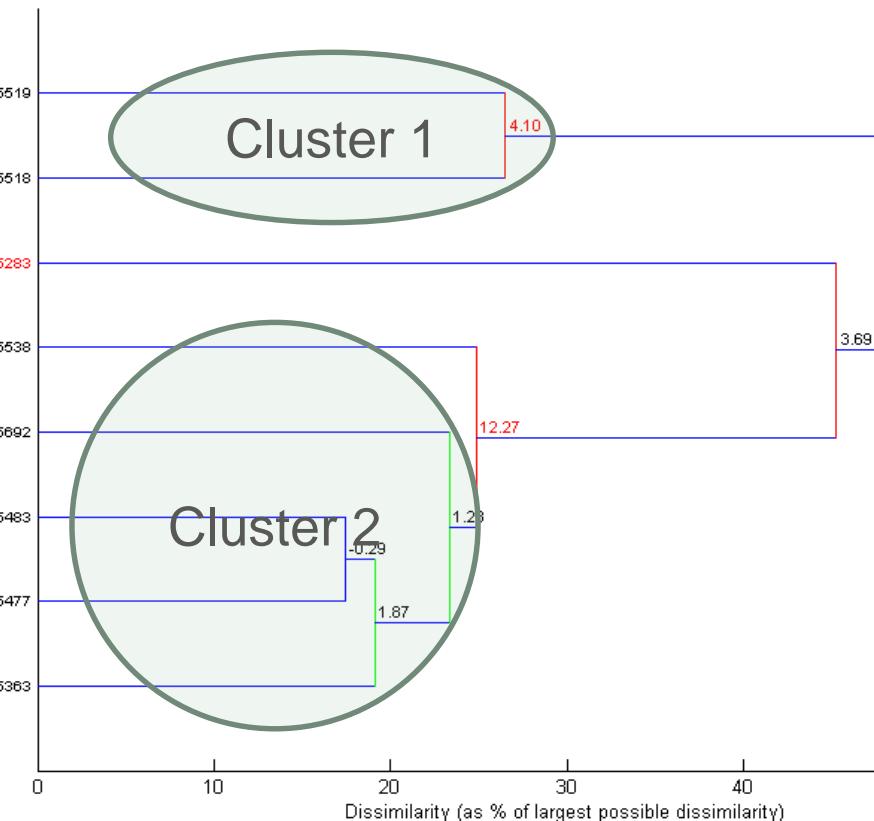
- Hemolysis assays
- Cross-species activity



# Identifying Clusters of Leads Combining Sequence Information and Screening Assay Data

Functional activity (hemolysis)

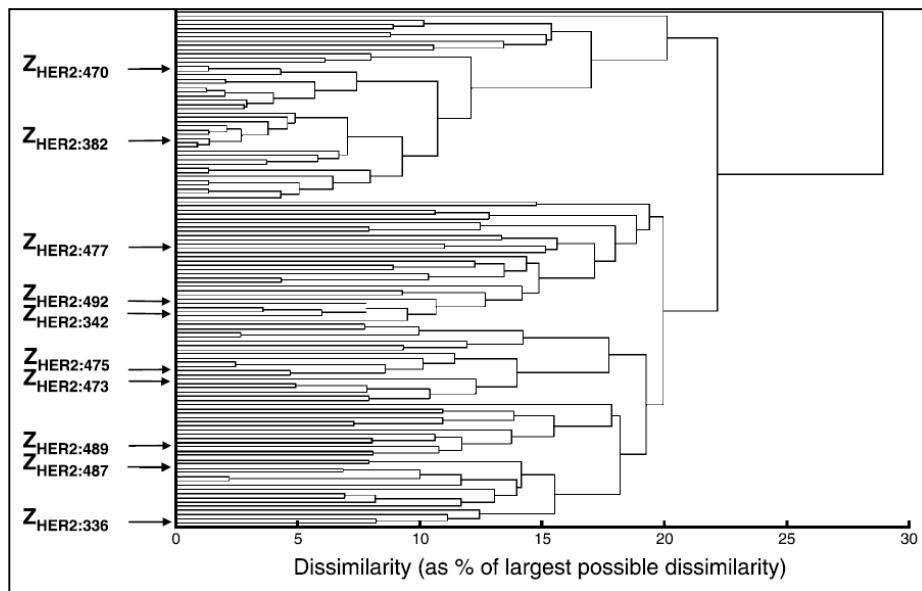
Affibody ligand	Human	Cyno	Mouse	Rat
Z0nnnn	+	++	-	(+)
Z0nnnn	+	+	-	-
Z0nnnn	+++	-	-	-
Z0nnnn	+	++	++	++
Z0nnnn	++	+++	-	++
Z0nnnn	++	++	(+)	(+)
Z0nnnn	++++	++++	++++	++++
Z0nnnn	(+)	(+)	-	(+)



# Lead optimization

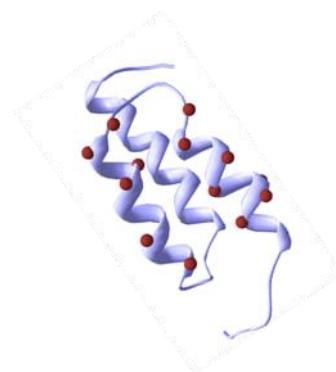
- In biopharmaceutical development this translates to optimization of the sequence of your protein
  - Increase affinity
  - Improve stability of protein
    - Structural
    - Chemical
  - Reduce immunogenicity
- Extend repertoire of properties by fusion with partner
  - Half-life
    - E.g. Albumin, IgG Fc, ABD
  - Add second activity
    - E.g. Bispecific antibodies

# Maturation of the Affibody molecules – Optimizing the affinity and other important properties of the Affibody molecules



**Maturation design based on function and properties of Primary Affibody® molecules**

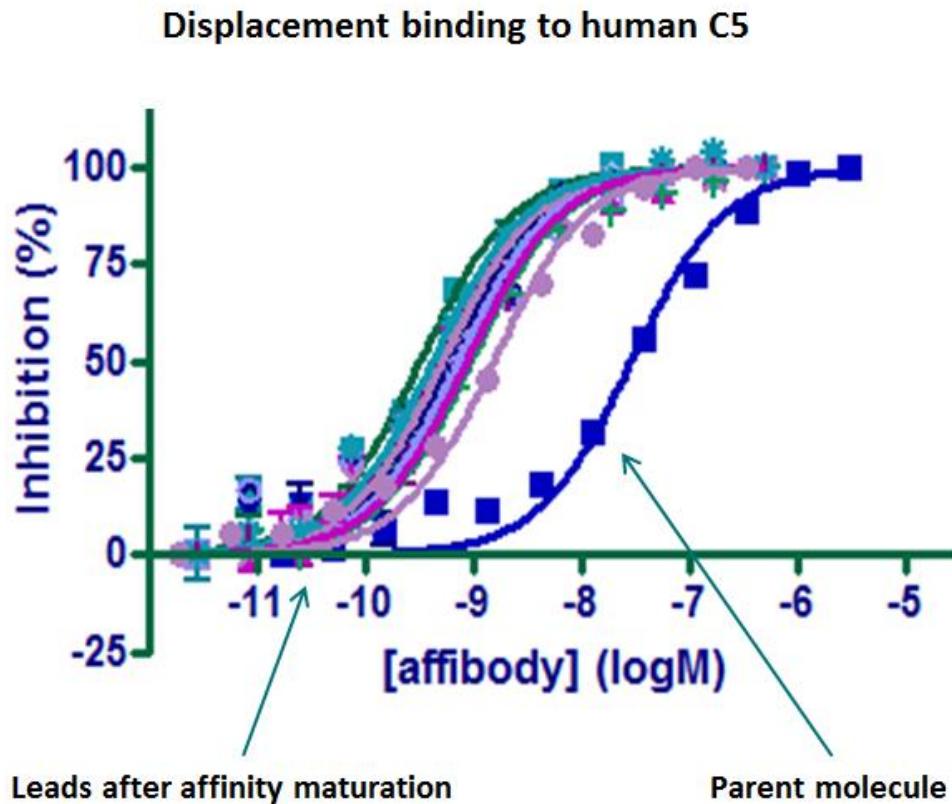
- Cluster analysis is used to group Affibody molecules based on their aa sequences' similarity/dissimilarity



	Helix 1	Helix 2	Helix 3				
Z <sub>wt</sub>	VDNKFNK	EQQNAFYEILH	LPNLNE	EQRNAFIQSLKD	DPSQ	SANLLAEAKKLND	QAPK
Z <sub>HER2:4</sub>	-----	-LRQ-YW--QA	-----W	T-SR---R--Y-	-----	-----	----- 33
Z <sub>HER2:7</sub>	-----	-PKT-YW--VK	-----P	E-RR---R--Y-	-----	-----	----- 5
Z <sub>HER2:24</sub>	-----	-PRE-YW--QR	-----N	K-KA---R--Y-	-----	-----	----- 1
Z <sub>HER2:79</sub>	-----	-WMT-GK--YR	-----G	T-VR---Q--S-	-----	-----	----- 1
Z <sub>HER2:2</sub>	-----	-WVQ-GS--YN	-----R	A-MR---R--S-	-----	-----	----- 2
Z <sub>HER2:8</sub>	-----	-IKQ-FH--VR	-----A	D-VR---Y--G-	-----	-----	----- 6
Z <sub>HER2:25</sub>	-----	-MVD-GA--WR	-----A	K-M*---D--G-	-----	-----	----- 1
Z <sub>LibHER2:mat</sub>	-----	-X <sup>RO</sup> <sub>KT</sub> -YW--XX	-----X	X-XR---R--Y-	-----	-----	-----

→ The Affibody molecule in the ABY-025 clinical product was selected from this library

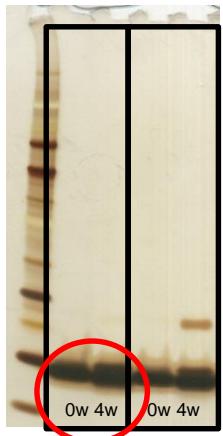
# Affinity Maturation Increased Affinity 100-fold



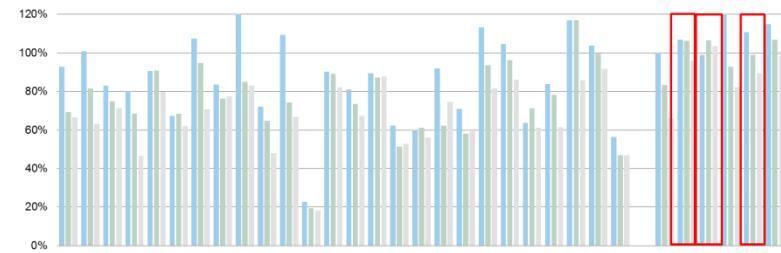
# Accelerated Stability Study

- Integrity after 4 weeks at 40°C (comparing 0, 2 and 4 weeks samples)

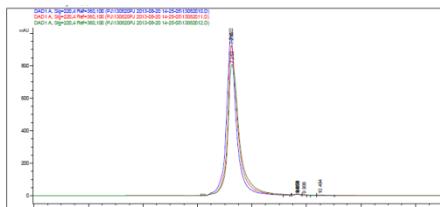
Gel: Silverstaining



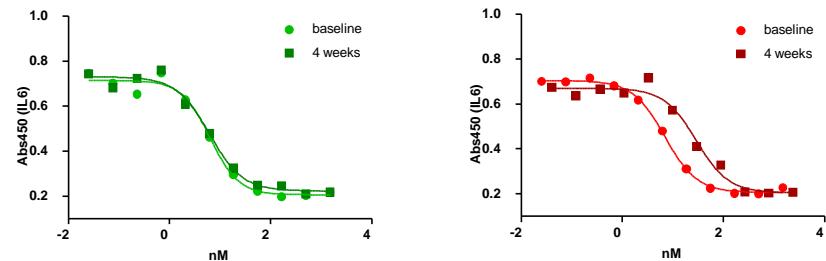
ELISA: Target binding



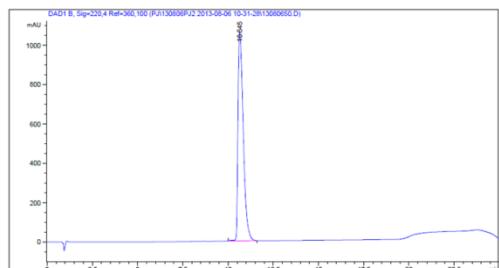
SEC: No aggregation



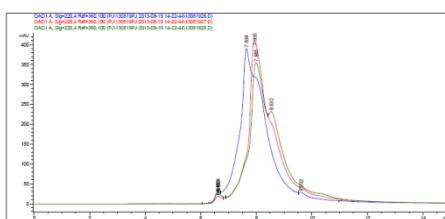
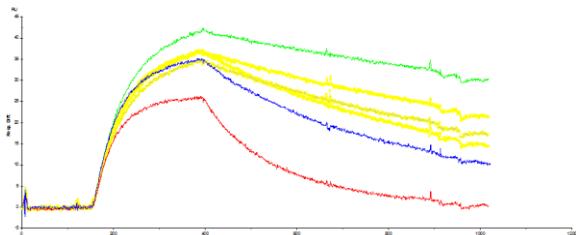
Blocking activity



LC/MS: No degradation

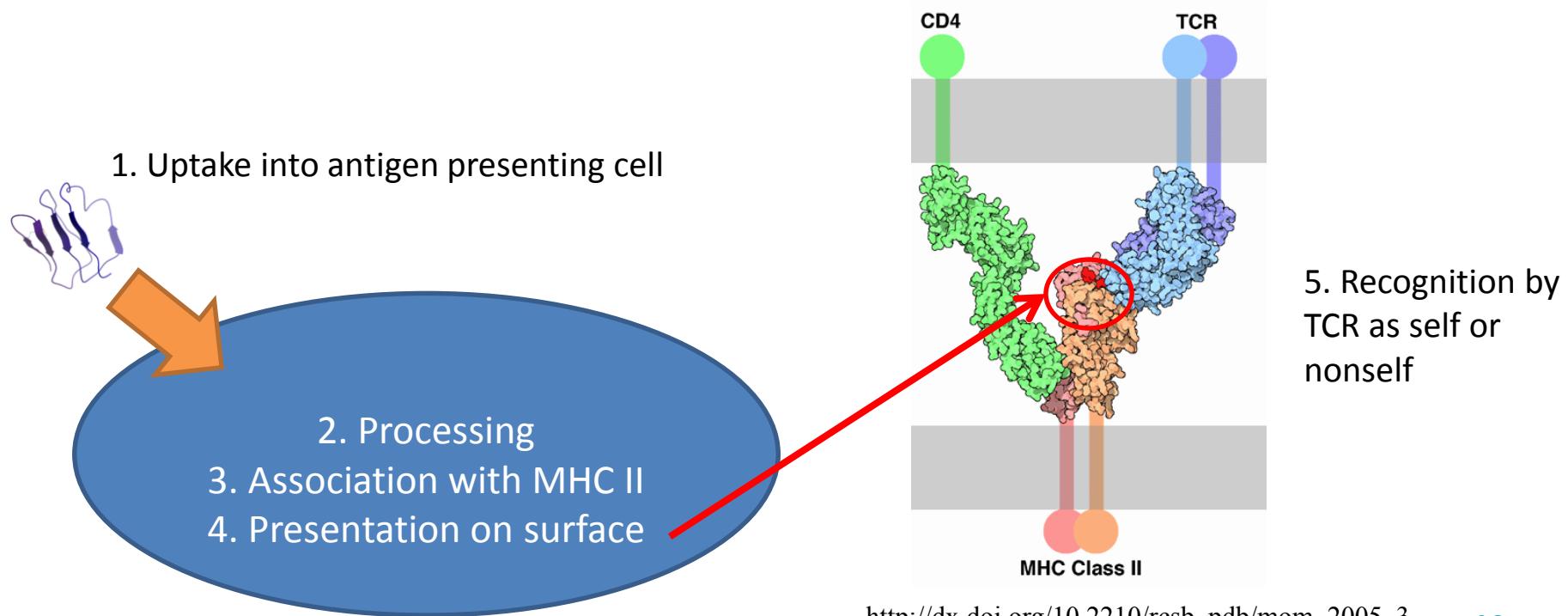


Biacore: Affinity



# Immunogenicity of biologicals

- Proteins do not share the same risk profile as small molecules that may interfere with a multitude of the functions in the body
- They may however induce an immuno response as the body has an excellent recognition of foreign biological material



# MHC binding prediction

**Table 1 Overview of human MHC class II loci, allele and polymorphism.**

Locus	Gene	Chain	# of alleles
HLA-DP	HLA-DPA1	alpha	28
HLA-DP	HLA-DPB1	beta	138
HLA-DQ	HLA-DQA1	alpha	35
HLA-DQ	HLA-DQB1	beta	108
HLA-DR	HLA-DRA	alpha	3
HLA-DR	HLA-DRB1	beta	785
HLA-DR	HLA-DRB2	beta	1
HLA-DR	HLA-DRB3	beta	52
HLA-DR	HLA-DRB4	beta	14
HLA-DR	HLA-DRB5	beta	19
HLA-DR	HLA-DRB6	beta	3
HLA-DR	HLA-DRB7	beta	2
HLA-DR	HLA-DRB8	beta	1
HLA-DR	HLA-DRB9	beta	1

Information was extracted from IMGT database. HLA-DM and HLA-DO molecules are not included as they are not expressed on cell surface.

- 22 318 peptides that binds to MHC class II with known affinity towards alleles
- Calculate properties of peptides
- Create prediction model for each allele
  - Machine learning algorithms like SVM and neural networks are most common

# Predict immunogenic sites

Allele	#	Start	End	Length	Peptide	Method used	Percentile rank
HLA-A*23:01	1	4	12	9	TWASDFERT	Consensus (ann/smm)	10.25
HLA-A*23:01	1	12	20	9	TERSSSWEL	Consensus (ann/smm)	11.8
HLA-A*23:01	1	15	23	9	SSSWELLKI	Consensus (ann/smm)	13.0
HLA-A*23:01	1	14	22	9	RSSSWELLK	Consensus (ann/smm)	17.0
HLA-A*23:01	1	16	24	9	SSWELLKIL	Consensus (ann/smm)	17.5
HLA-A*23:01	1	1	9	9	EERTWASDF	Consensus (ann/smm)	18.0

Response to prediction:

- Select lowest scoring among leads
- Create variants to diminish response
  - Iterate through prediction

EERTWASDFERTERSSSWELLKILKK  
**EERTWASDF**  
ERTWASDFE  
RTWASDFER  
**TWASDFERT**  
WASDFERTE  
ASDFERTER  
SDFERTERS  
DFERTERSS  
FERTERSSS  
ERTERSSSW  
RTERSSSWE  
**TERSSSWEL**  
ERSSSWELL  
**RSSSWELLK**  
**SSSWELLKI**  
**SSWELLKIL**  
SWELLKILK  
WELLKILKK



EERT**WASDF**ERTERS**SSWEL**LKILKK

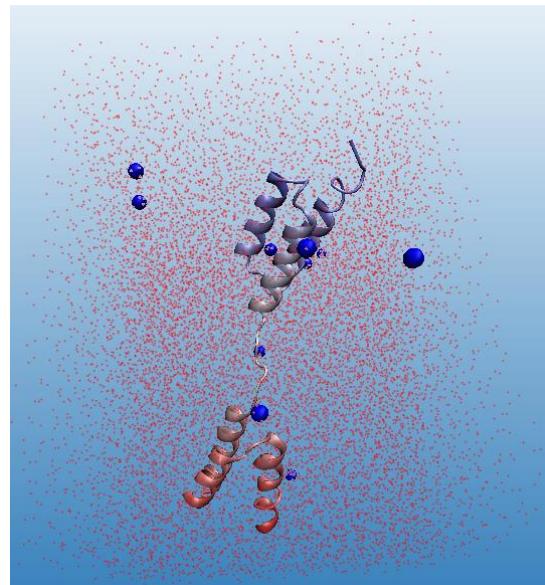
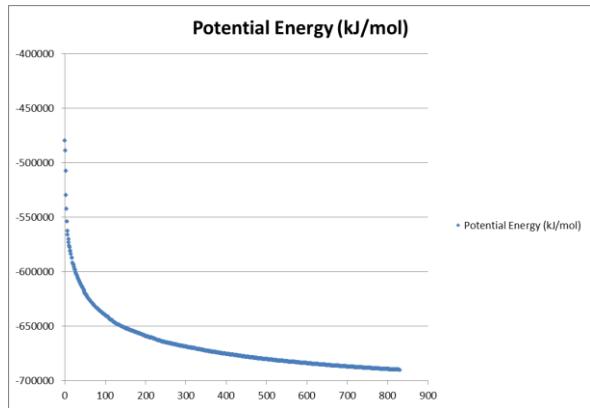
# Guide for protein engineering

- Molecular dynamics simulation to assess effect of sequence upon structure
- Molecular movements and their time scales

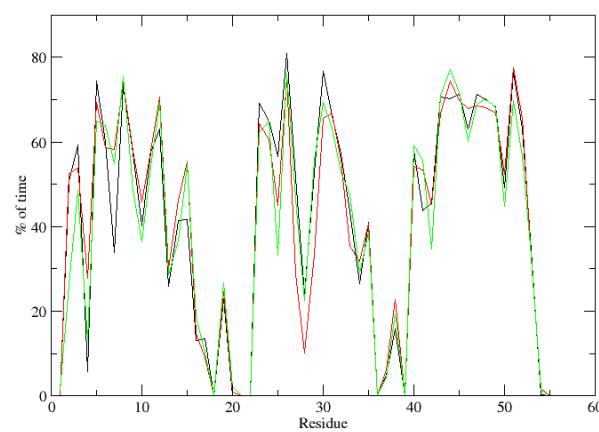
Time scale	Amplitude	Description
short femto, pico $10^{-15} - 10^{-12}$ s	0.001 - 0.1 Å	-bond stretching, angle bending -constraint dihedral motion
medium pico, nano $10^{-12} - 10^{-9}$ s	0.1 - 10 Å	-unhindered surface side chain motion -loop motion, collective motion
long nano, micro $10^{-9} - 10^{-6}$ s	1 - 100 Å	-folding in small peptides -helix coil transition
very long micro $10^{-6} - 10^{-1}$ s	10 - 100 Å	-protein folding

# MD introduction

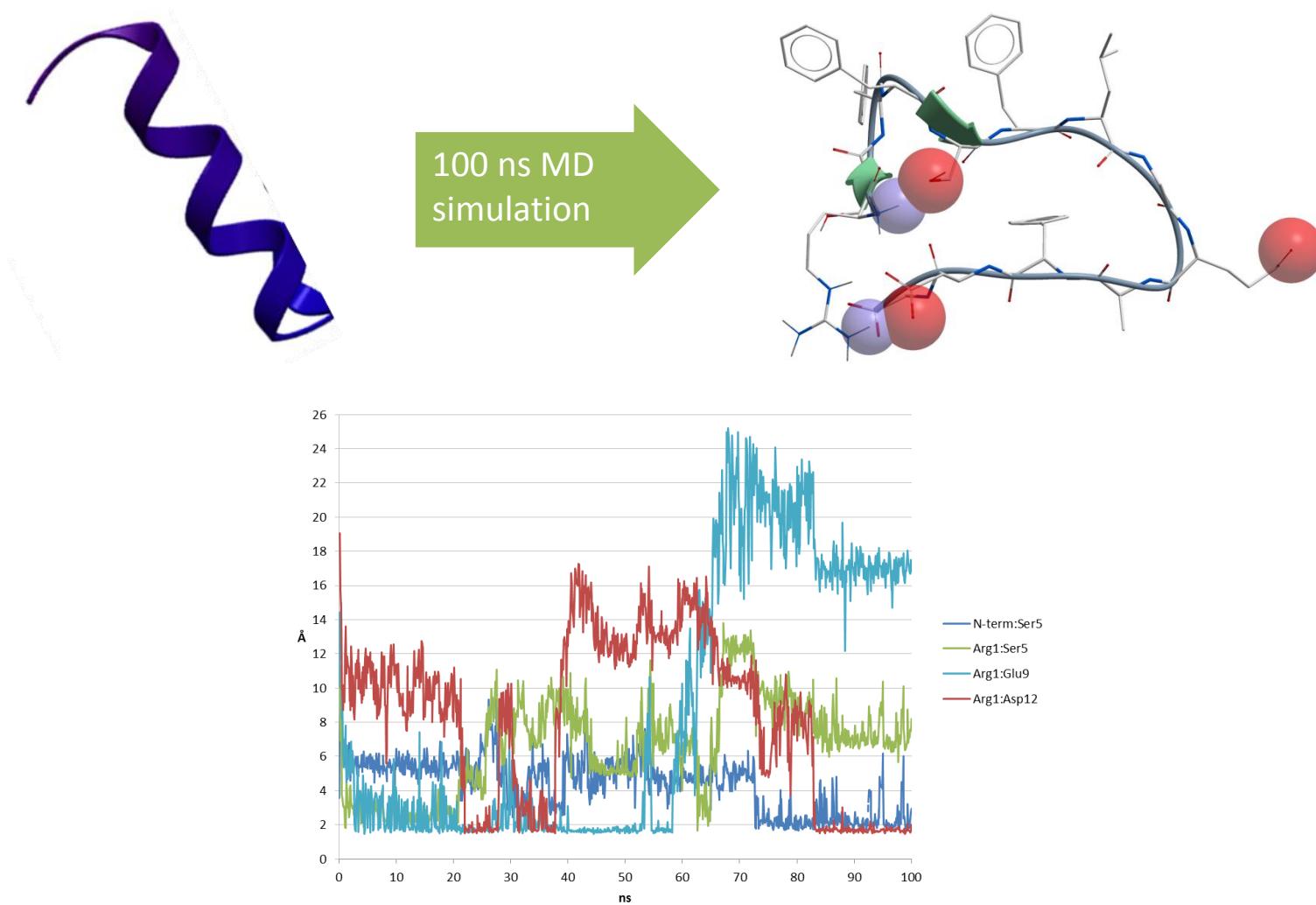
- Prepare system with water and counterions
- Equilibrate system with restraints



- Perform analytical calculation
  - Analyse trajectory



# Monitor interactions through simulation

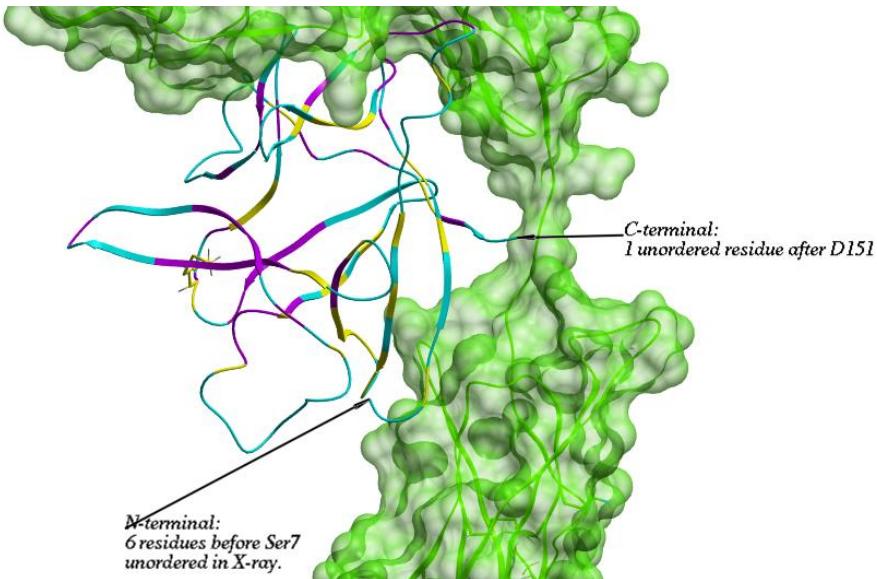


# MD summary

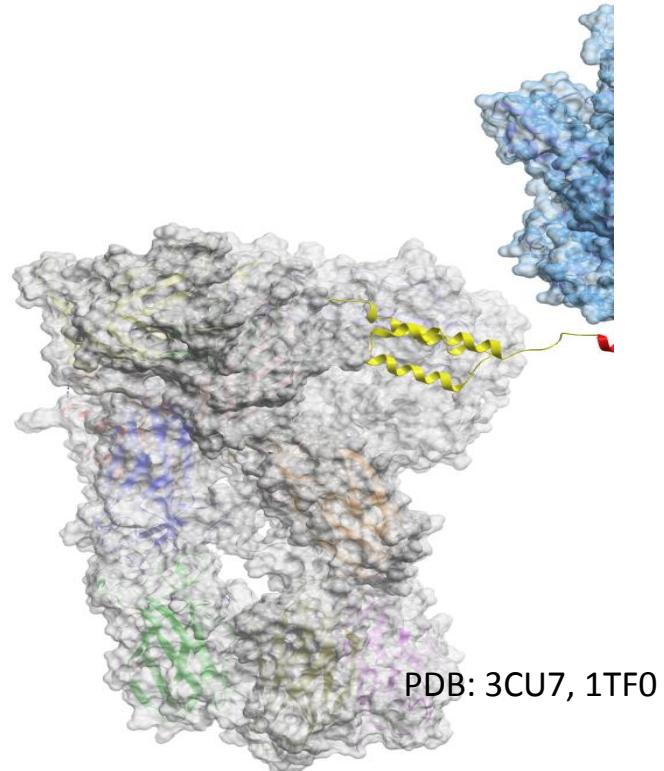
- Provide information about flexible region of proteins and interdomain motions
  - Suitable for optimisation of fusion proteins
- Influence of amino acid exchanges upon structural integrity
  - Suitable as filter for sequences to express
- Can provide molecular hypothesis for observed transitions

# Fusion protein design introduction

- Order of domains
  - Structural consideration
  - C-terminal probably requires a linker to maintain affinity of IL-1Ra
- Linker lengths
  - Allow target to be engaged without loss of activity



PDB: 1IRA

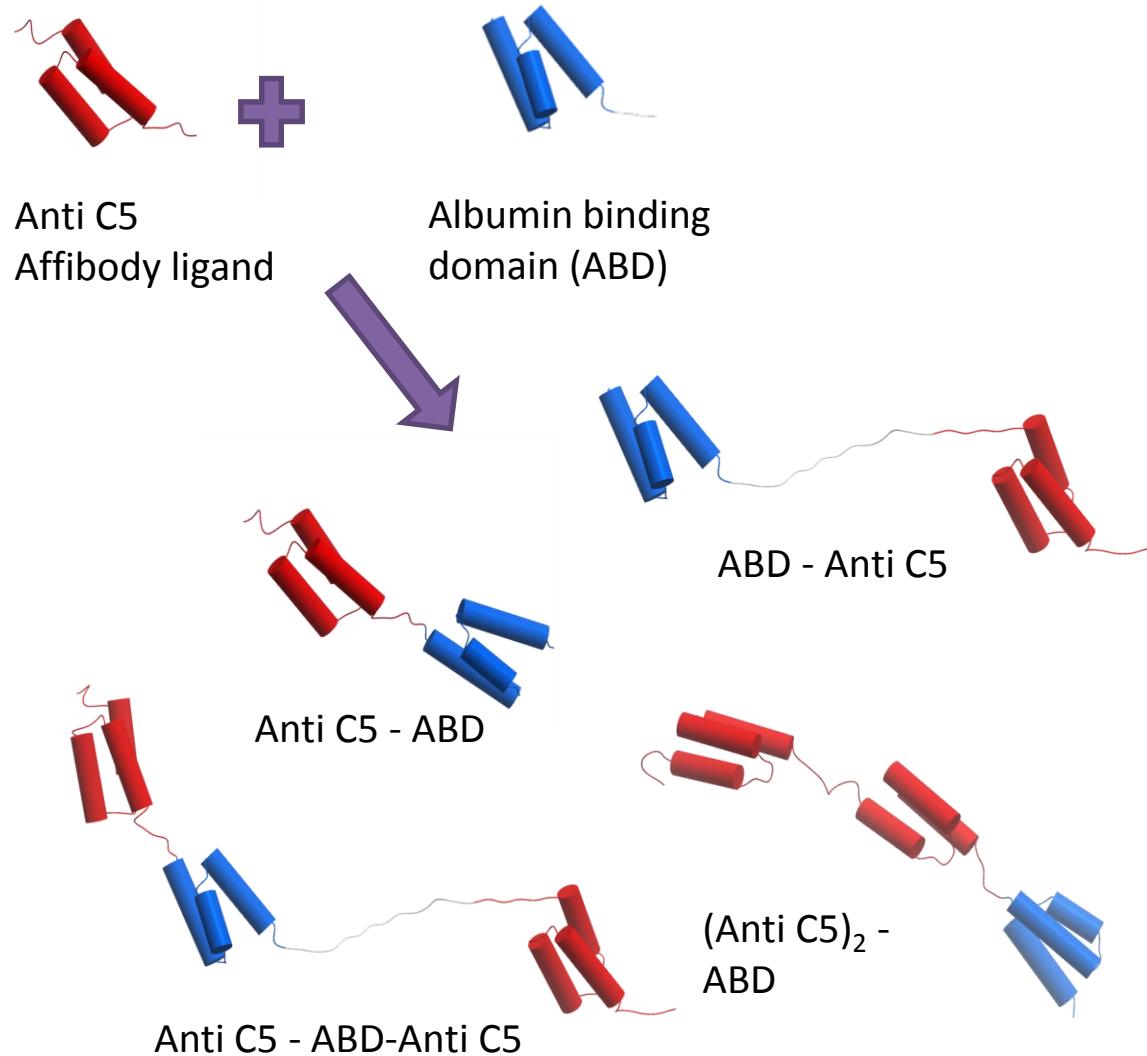


# Case Study Lead Optimization

## Designing the Fusion Protein

### Variables:

- C5 binding domain (shortlist)
- Order of domains
- Multiple C5-binding domains
- Different linkers
- etc.



### Assessments:

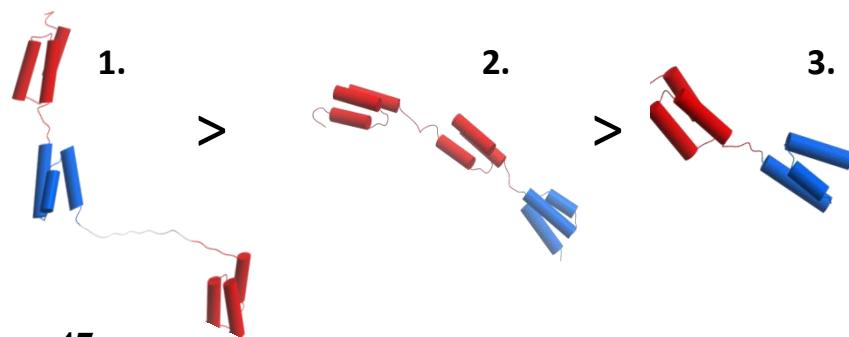
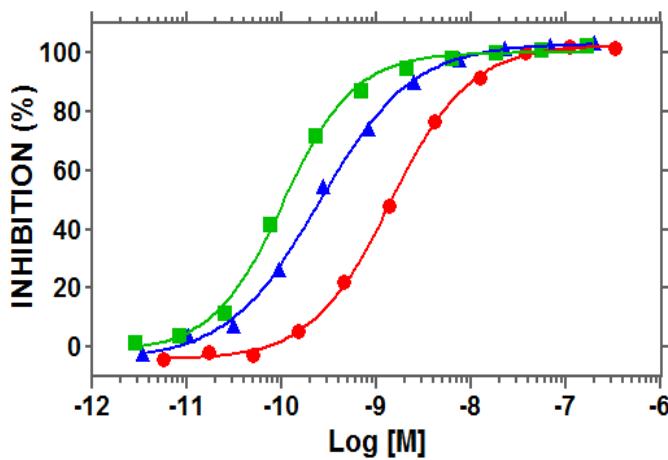
- *In vitro* pharmacology
- Physicochemical properties
- Immunogenicity assessment
- *In vivo* PK/PD

# Lead Optimization - Selecting the Best Design

## i. *In vitro* activity

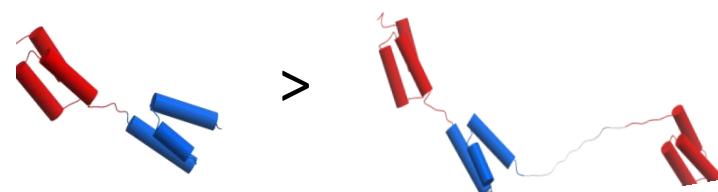
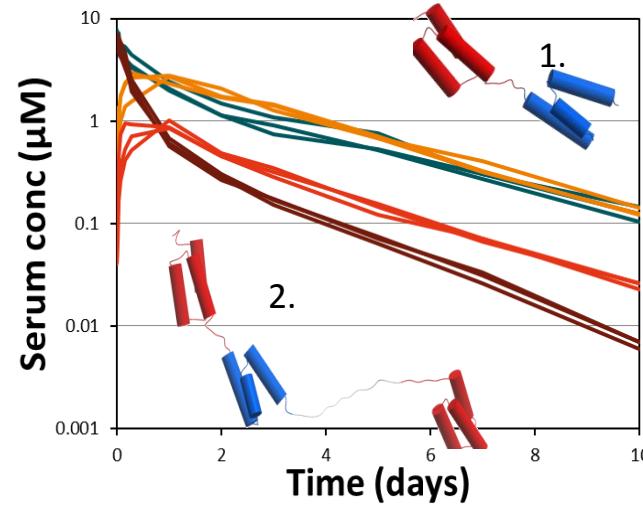
- Hemolysis assays

Hemolysis assay



## ii. Pharmacokinetics in rodents ▪ i.v. and s.c.

Rat PK study



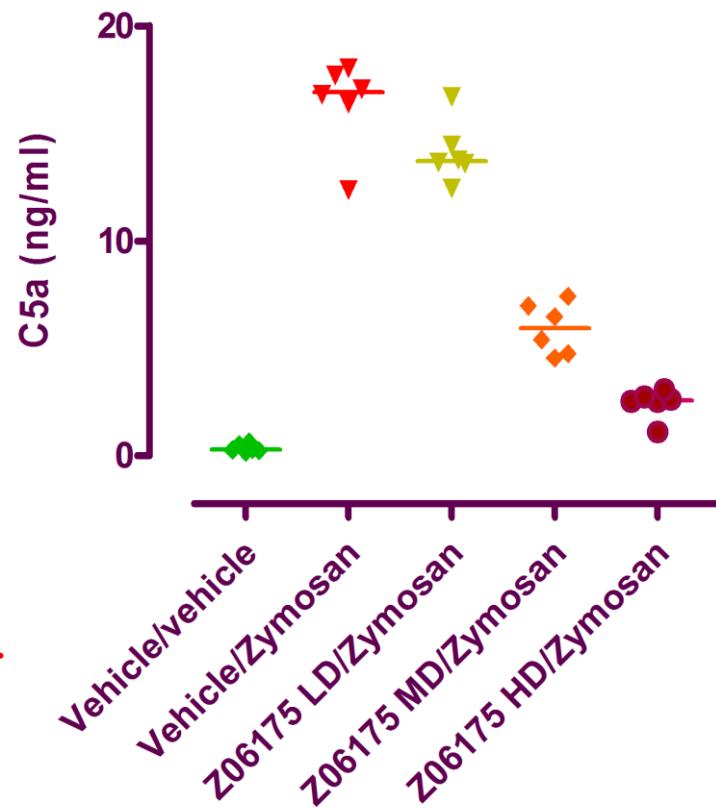
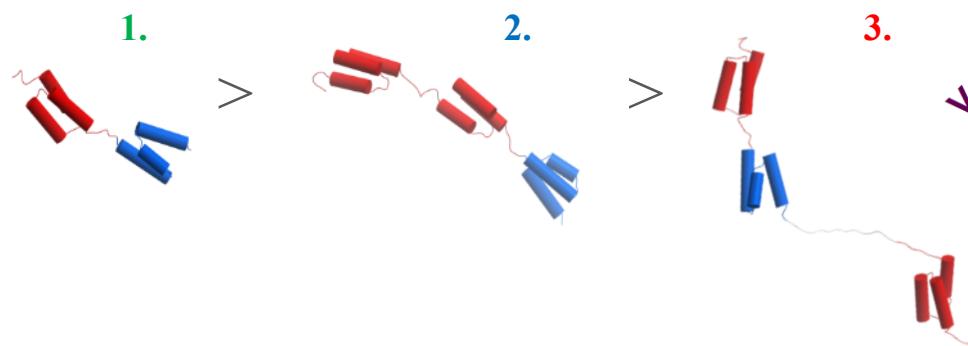
# Lead Optimization - Selecting the Best Design

## *In vivo* activity

Acute mouse inflammation model

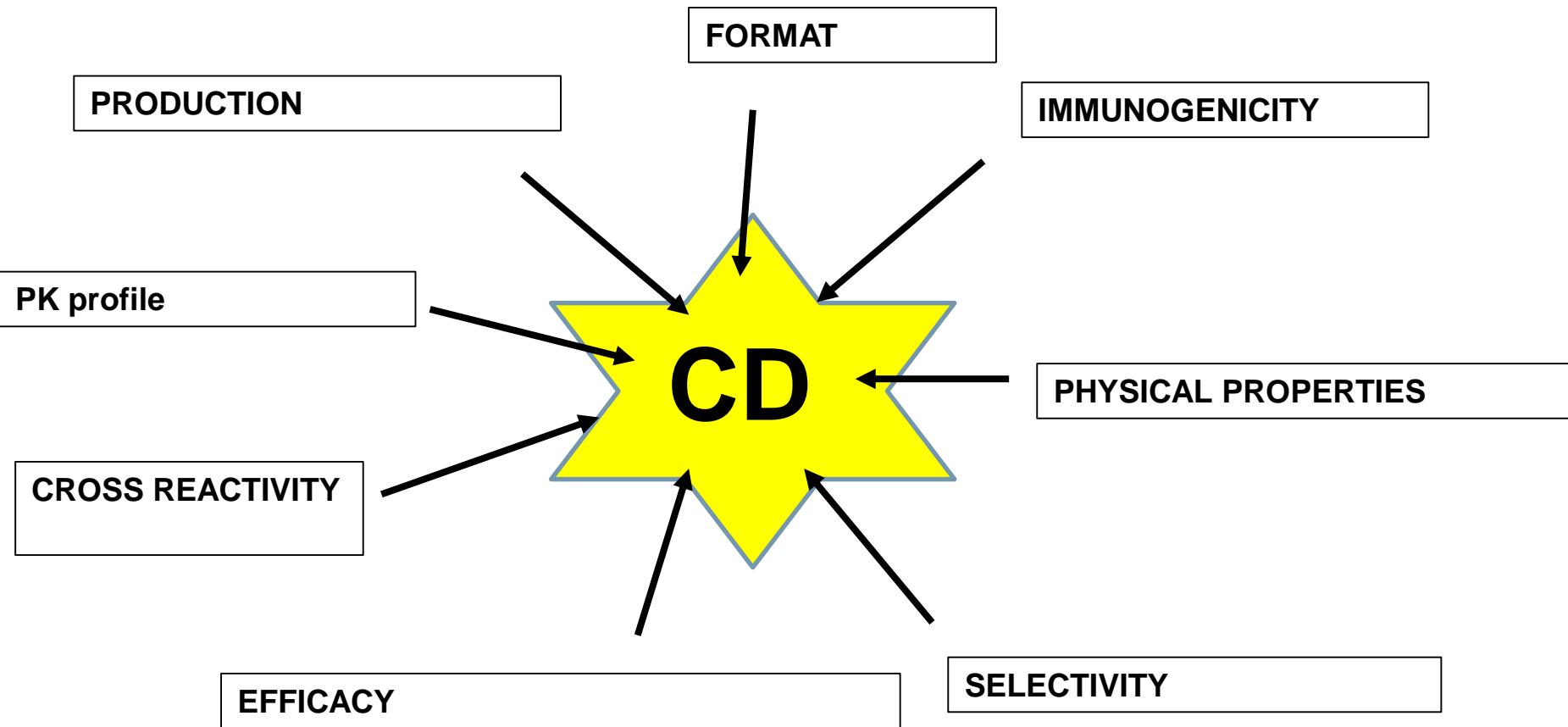
Zymosan induced peritonitis

C5a in peritoneal lavage



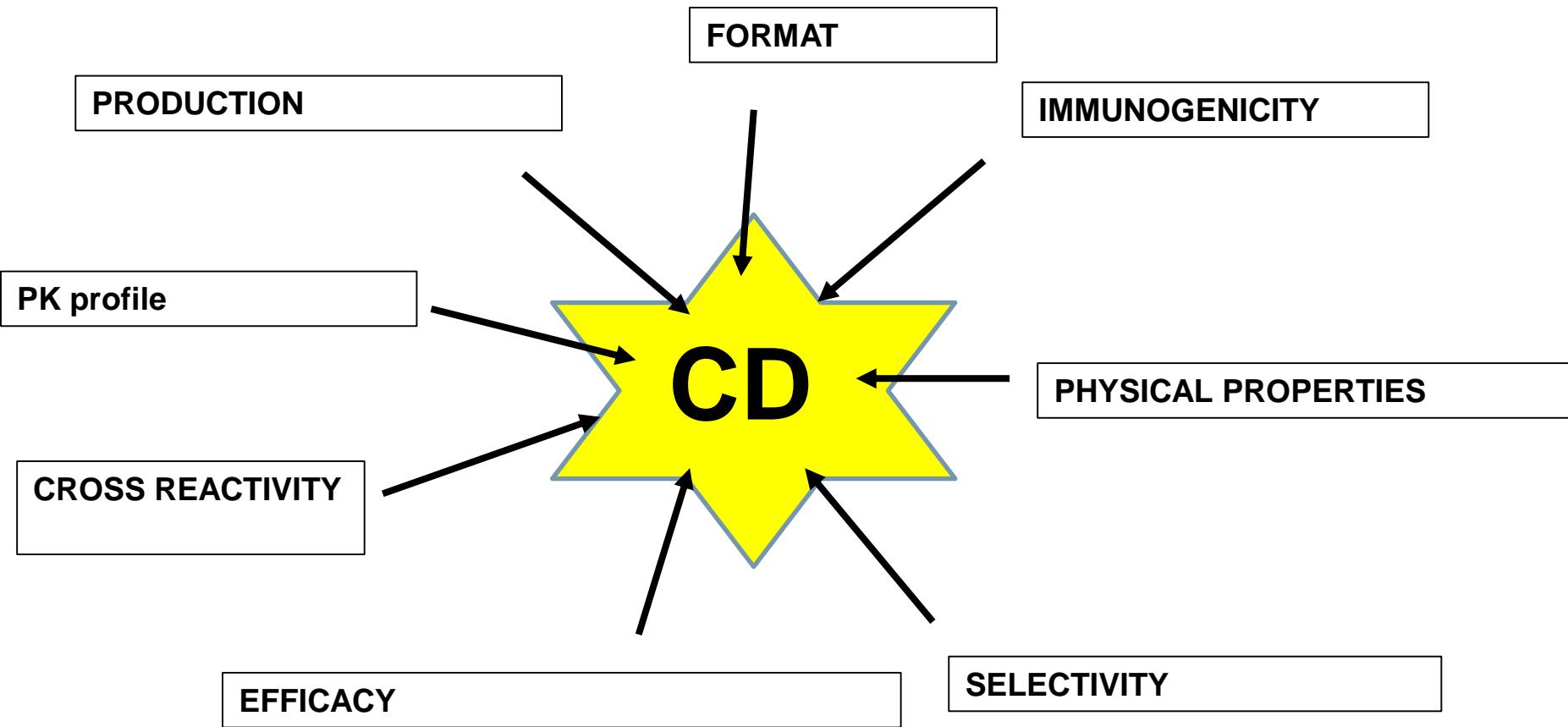
## **CD criteria**

– *to be set within the project to allow CD nomination*



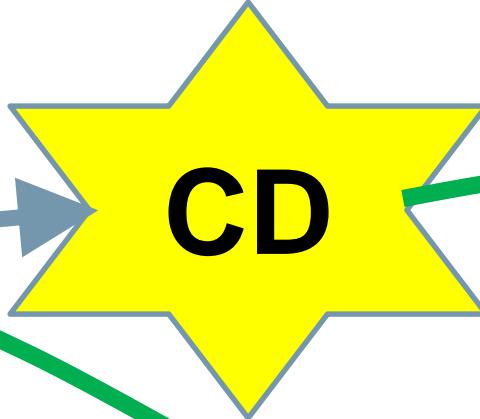
## CD criteria

– to be set within the project to allow CD nomination



What favourite property would YOU select to optimize and why?

- GLP manufacturing
- Toxicity Studies
- GMP Manufacturing
- CTA/IND
- Phase I – II – III Studies
- MAA





Pioneer in Rare Diseases



**THANK YOU!**